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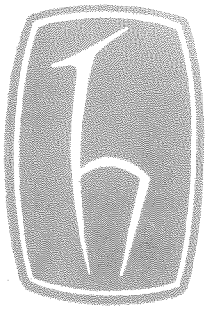

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Alpha-Feto Protein in Viral Hepatits and in the Cirrhosis of Liver

Hasan Ünal, M.D.* / Abdulahad Taher, M.D.**
Şükran Karacadağ, M.D.***

Alpha-feto protein (AFP) is a normal fetal serum alfa globulin that is synthesized by embryonal liver cells, and reaches the maximal concentration between 12-16 weeks of fetal life. It disappears from the serum within days to weeks after birth and reappears in the serum of patients with hepatocelluler carcinoma, [1, 2, 3] testicular and ovarian tumors,[4] metastatic liver disease[5, 6] and in non-neoplastic diseases.[7, 8]

There are few reports concerning the AFP in viral hepatitis and in the cirrhosis of liver.[4,9-12] In this paper we report the incidence of AFP in 38 patients with viral hepatitis and in 22 patients with cirrhosis of the liver.

Material and Methods

AFP were determined in there groups :

I. Infectious Hepatitis: There were 38 patients. The diagnosis was based on case history, physical examination and classical liver function tests. For all patients Au antigen determination by immunodiffusion technique was carried out.[13]

II. Cirrhosis of the Liver: There were 22 patients. whose diagnoses were based upon medical history, physical findings, liver function tests and liver biopsy.

III. Control Group: There were 24 healthy controls, with no history of previous liver disease.

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Sera from these 3 groups were tested for AFP both by Ouchterlony double immunodiffusion in agar gel[13] and counter-immunoelectrophoresis[14] techniques.

Results

Results are shown in Table I. Only 3 patients with viral hepatitis had positive AFP, and this was confirmed by the two different methods. No difference was found between the double-gel diffusion and counter-immunoelectrophoresis techniques.

TABLE I

	Number of Patients	AFP Positive Cases
I. Viral Hepatitis	38	3
Au –	22	2
Au +	16	1
II. Cirrhosis of the Liver	22	0
Portal Cirrhosis	18	0
Post necrotic Cirrhosis	4	0
III. Normal Controls	24	0

Discussion

The presence of AFP in adults by immunodiffusion was considered almost to be a diagnostic test for primary liver cell carcinoma. But, later it was found positive in other tumors and nonneoplastic diseases. Recently AFP determinations have been performed by the radio-immunoassay method and the presence of small amounts of AFP were reported in normal adults. However, it is not possible to determine such a small amount of AFP by immunodiffusion and counter-immunoelectrophoresis methods. We found no difference between immunodiffusion in agar gel and counter-immunoelectrophoresis techniques. The same results were reported by Foli and Sherlock.[9] But, the general feeling is that counter-immunoelectrophoresis is more sensitive than immunodiffusion in agar gel. The antiserum pool could detect as little as 1 mg per 100 ml of AFP by Ouchterlony double immunodiffusion. The counter immunoelectrophoresis method increased the sensitivity 40 fold to a lower limit of approximately 0.025 mg per 100 ml.[15] Now, it is a well known fact that the best method is the radioimmunoassay method.

In our study, AFP was found negative in 22 patients with cirrhosis of the liver. Similar results were reported by others.[9, 10]Rarely is AFP found to be positive in cirrhosis of the liver.[1-4]

In this study only 3 patients with viral hepatitis had positive AFP. All patients had AFP determination when transaminases were at a high level. Unfortunately no follow-up studies were performed on our patients. Smith[11] performed AFP determinations in 11 patients with Au (+) and in 11 patients who had Au (—) hepatitis. AFP was positive in the active stage of the disease and disappeared when the liver function tests returned to normal with the dissappearance of Au antigen. Masopus, et al[10] found the presence of AFP in 10 out of 16 infants with infections hepatitis and it dissappeared with the regression of the disease. AFP was found to be negative in all children and adults with infectious hepatitis. Buffe and Rimbaut[16] found that 13 of 40 children (33 %) with acute hepatitis had AFP in their sera, although only transiently up to 75 days from the onset. This relatively high incidence of AFP positivity in children with acute hepatitis is interesting. In contrast to these studies others[1-9] showed the absence of AFP in infections hepatitis. It can be said that in some patients with infections hepatitis the AFP could be positive and the liver cell regeneration might be responsible for this. However, others[11] showed the presence of AFP only in patients with Au + hepatitis and concluded that the Au antigen might cause the AFP production or disturb repressor mechanisms. We found no definite relation between the Au antigen and the presence of AFP.

Summary

Using double immunodiffusion in agar-gel and counter-immunoelectrophoresis techniques AFP determinations were performed in 38 patients with viral hepatitis and 22 patients with cirrhosis of the liver AFP was found positive in 3 patients with viral hepatitis. All patients with cirrhosis of the liver had negative AFP in their sera.

No difference was found between the double gel diffusion and counter immunoelectrophoresis techniques.

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A Mechanical Method to Control the Extrasystoles of Ventricular Origin

Coşkun İkizler, M.D.* / Aydın Aytaç, M.D., F.A.C.C., F.A.C.S.**
Rustem Olga, M.D.***

A new study on the prevention of ventricular extrasystoles and a new mechanical method of practical importance, especially because of its simple application, is described. A preliminary report with obtained results is presented for this purpose.

Extrasystoles or premature beats are cardiac contractions of ectopic origin preceeding the dominant rhythm.[1] The active impluse is focused in the ventricles in ventricular premature beats. They usually originate in the His bundle or in the subendocardial plexuses of purkinje fibers. Numerous agents are used with reference to the treatment of ventricular extrasystoles with various results. In addition, a mechanical method is proposed by Barker, in which the ventricular extrasystoles are produced by the two-step exercise (Master) test.[2] By further increasing the heart rate with exercise, disappearance of the extrasystoles are seen in some cases. However, the impossibility of the application of this method for the majority of patients, especially surgical cases, led us to develop the new mechanical method described in detail below. Our study is not an etiologic investigation of extrasystoles.

Materials and Method

This maneuver has been applied to some of the patients *during* or *after* open heart surgery at the clinic. In Table I the data for the 27 cases upon whom the mechanical method was applied between November, 1969, and January, 1973. are shown.

* Lecturer in Pediatric Thoracic and Cardiovascular Surgery.
** Professor of Cardiovascular Surgery and Chief of Pediatric Thoracic and Cardiovascular Surgery
*** Lecturer in Pediatric Thoracic and Cardiovascular Surgery.

TABLE I

Diagnosis	Operation	Number of Cases
AS-AI	AVR	6
MS-MI-TI	MVR-Tricuspid Valvuloplasti	7
MS-MI, AS-AI	MVR-AVR	12
TOF	Total Correction	1
MS-MI-TI with digitalis intoxication	No operation	1
Total		27

Abbreviations: AS-Aortic Stenosis, AI-Aortic Insufficiency, MS-Mitral Stenosis, MI-Mitral Insufficiency, TI-Tricuspid Insufficiency, ToF-Tetralogy of Fallot, AVR-Aortic Valve Replacement MVR-Mitral Valve Replacement.

This maneuver has been applied to extrasystoles seen during open heart surgery, when the heart began to beat after the elective cardiac arrest produced by the aortic clampage or during the postoperative period. In additon, the maneuver has been applied to one of the cases with digitalis intoxication in the emergency room prior to the operation.

Application of the maneuver

a. During the operation: (Figure 1) If premature beats are present, the right and left ventricles of the heart are grasped and gently compressed with the palms of both hands.

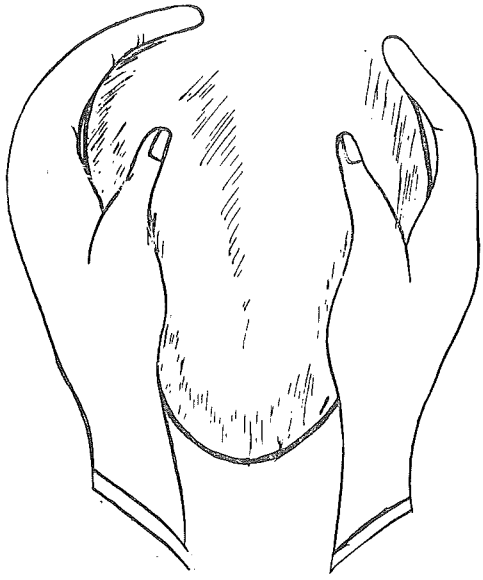


Figure 1
During the operation

b. During the pre-or post-operative period extrasystoles seen at the bedside: One stands at the right side of the patient with the palm of the right hand placed at the apex. While pulling the thorax to the right, pressure is applied so as to stabilize the heart between the mediastinum and the thoracic wall. At this moment, the left hand could be placed on the right shoulder of the patient (Figure 2).

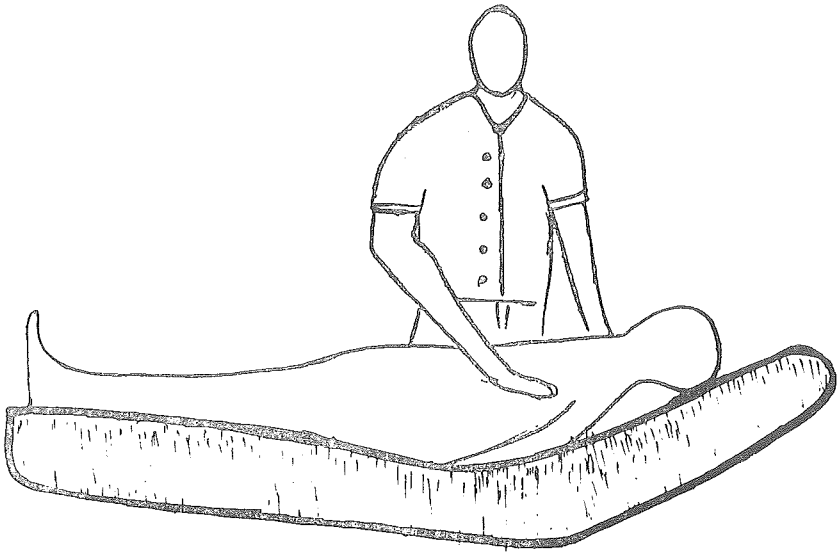


Figure 2
At the bed side

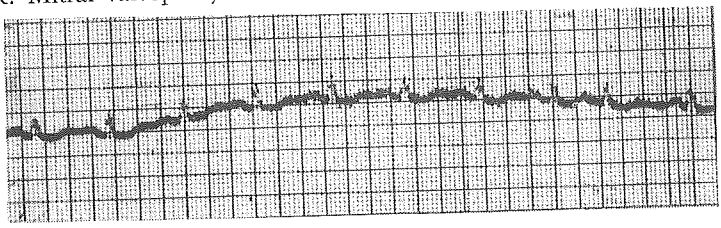
Results

This maneuver has been applied to patients with ventricular extrasystoles during operations or in the post-operative period of open heart surgeries; and upon Another which was applied was for digitalis intoxica tion. The intoxicated patient had taken six tablets of Natigoxin (0.25 mg per tablet) a day in spite of the completed full digitalization. We obtained successful results on 26 patients out of 27 upon whom we applied these maneuvers.

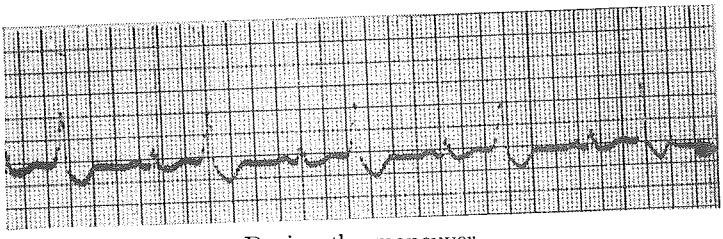
For 10 patients among the total number of operated cases, the maneuvers were applied twice, once during the operation and again in the postoperative period. We were not successful in three of 10 patients due to digitalis intoxication. (Figure 3a, 3b, 4a, 4b, 5a, 5b, 6, 7, 8).

M. L. 30 Years old ♀
67-29132
A. V. R. Mitral valvoplasty

Carte I



Before the maneuver

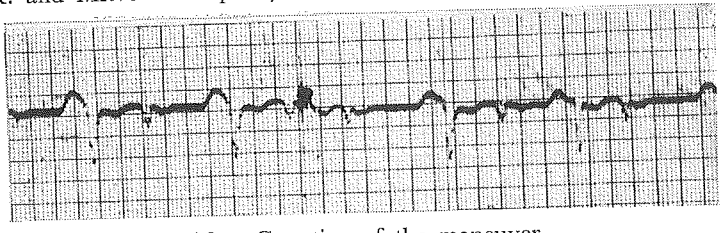


During the maneuver

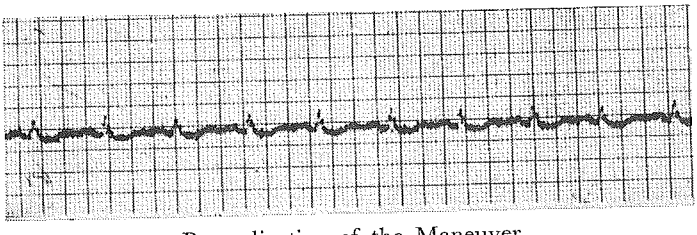
Figure 3a

M. L. 30 Years old ♀
67-29132
A. V. R. and Mitvol Valvoplasty

Carte II



After Cessation of the maneuver

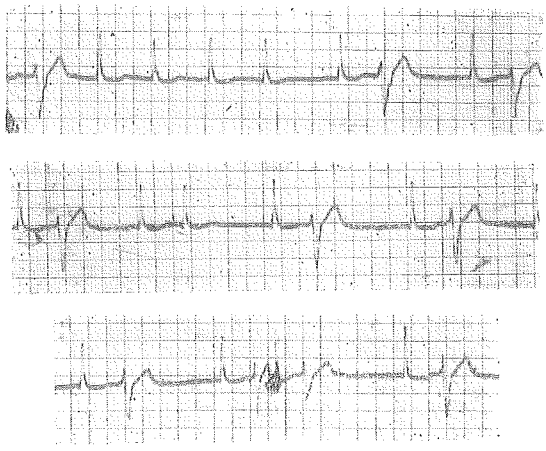


Reapplication of the Maneuver

Figure 3b

S. Ö. 19 Years old ♀
8 2Z03
M. V. R.

Carte I

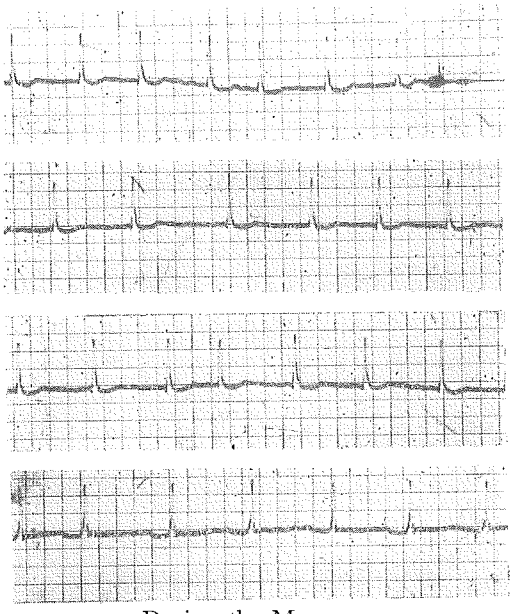


Before the Manevver.

Figure 4a

S. Ö. 19 Years old
82203
M. V. R.

Carte II

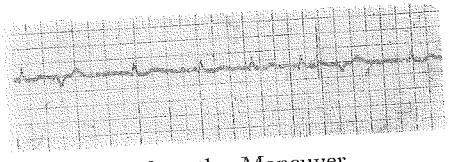


During the Maneuver

Figure 4b

M. S. 38 Years old ♀
97684
A. V. R.

Carte I



Before the Maneuver

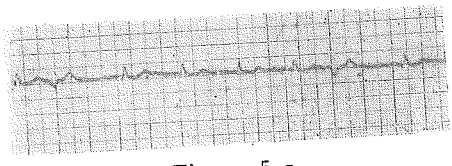
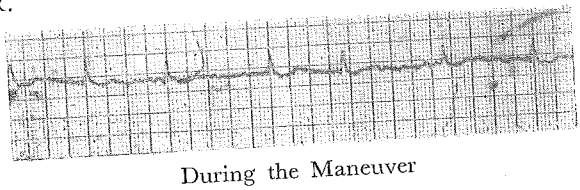


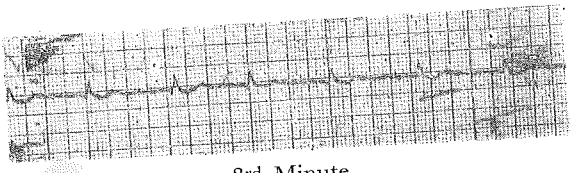
Figure 5 a

M. S. 38 Years old ♂
97684
A. V. R.

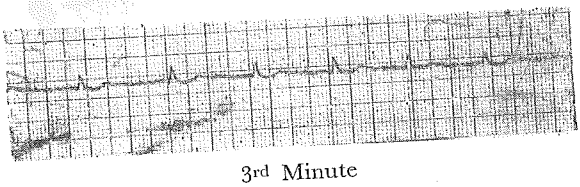
Carte II



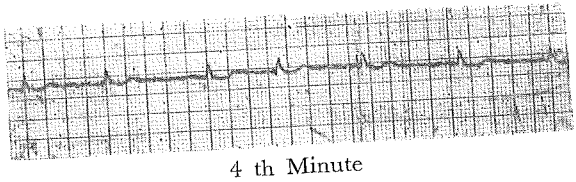
During the Maneuver



3rd Minute



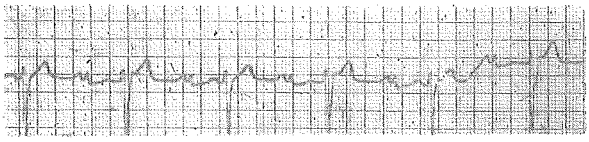
3rd Minute



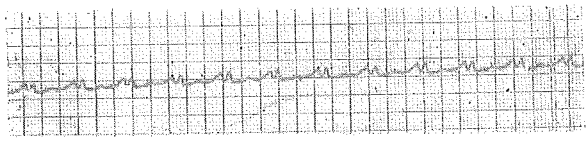
4 th Minute

Figure 5 b

B. C. 14 Years old
115444
M. V. R.



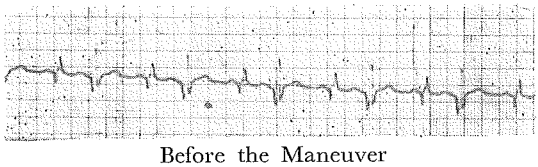
After the Maneuver



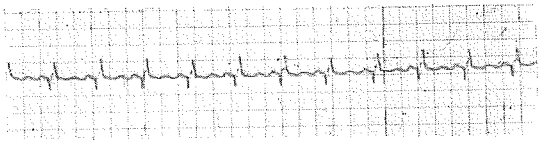
Before the Maneuver

Figure 6

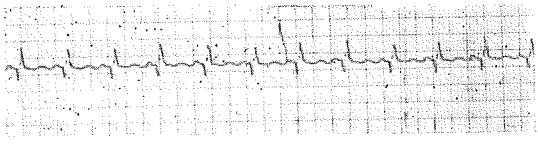
Y. E. 19 Years old
59-4498
A. V. R.



Before the Maneuver 1st Minute



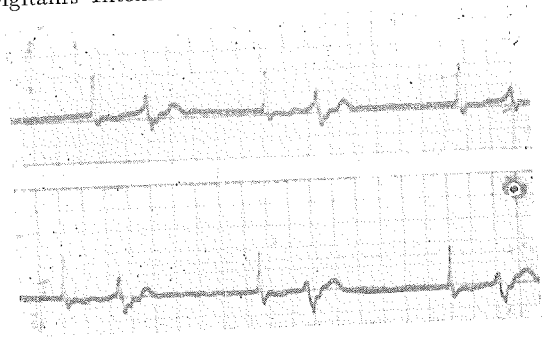
5th Minute



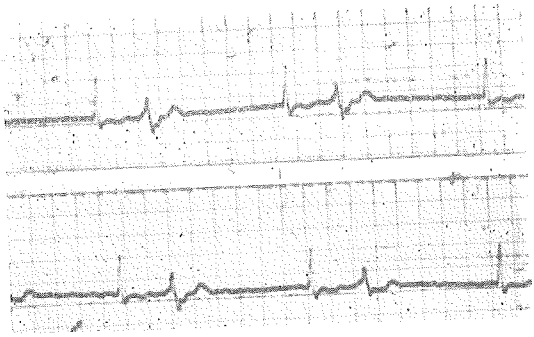
During the Maneuver

Figure 7

E. K. 19 Years old ♀
144066
M. I. + MS Digitalirs Intoxication



Before the Maneuver



During the Maneuver

Figure 8

Discussion

In all of the cases, the heart had begun to beat either spontaneously or by defibrilation. The myocardial metabolism began to improve after anoxic arrest with the removal of the aortic clamp. The contractability, which is already limited after all these phases, may become effective within a short or long time following the anoxic arrest through the gain of power of the myocardial fibers. The ectopic nodes causing extrasystoles are easily formed during this period.

The effectiveness of the maneuver is possibly related to the support of the heart on both sides, so that the atonic myocardium is more cooperative during the systole, and its overstretch and vibrations by the blood during the diastole are prevented.

During the maneuver, the extrasystoles disappear, but when the maneuver is discontinued the extrasystoles are seen again. The ineffect-

iveness of the maneuver on the patients with digitalis intoxication seem to suggest that the effectiveness is obtained in a mechanical way.

The settling of the non-extrasystolic rhythm, after the completion of the required period for the myocardium to regain its power, shows the usefulness of this mechanical method in supporting the myocardium during this period. Our method is practical in many aspects and it can be applied easily at the bedside with very satisfactory results. However, the external application is difficult in some cases who have a rigidly developed thoracic wall.

This preliminary report may motivate some new investigations and an instrument which can be used to apply this mechanical method may be developed.

Summary

A new mechanical method was described to control ventricular extrasystoles. This method has been applied to 27 cases, 26 of whom had had heart operations. All these patients had ventricular extrasystoles such as bigeminy, trigeminy and irregular types. Results being satisfactory, we are continuing the use of this method with success.

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Preliminary Report :

Elevated Glutathione Levels in Erythrocyte of Humans with Gastrointestinal Adenocarcinoma

Atilla Engin, M.D., Ph.D.*

Glutathione (GSH), plays a very important role in mature erythrocyte metabolism, by protecting the sulphydryl groups of different substances, such as hemoglobin, catalase and the lipoprotein of cell membranes.[1-5] Additionally, GSH is more readily oxidized than the protein sulphydryls, and it serves as a source of oxidizing power to reduce disulphilde bonds which are deleterious to red cell functions.[1, 4, 7] Although the precise role of GSH in many tissues is still obscure, GSH is present in relatively large amounts in the erythrocytes and the reduction of oxidized glutathione to reduced glutathione represents an important potential source of $NADP^+$ in red cells.[6, 7]

Some changes have been measured in the GSH levels of erythrocytes in a number of metabolic disorders, but there is no clear explanation about the cause and the origin of these elevations.[8, 9] Furthermore, no reports could be found in the literature concerning the GSH level of red cells of patients with advanced gastrointesitinal tract carcinoma.

Materials and Methods

The red blood cell GSH values were measured in one group of patients with histologically proven disseminated adenocarcinomas of the gastrointestinal tract. Erythrocytes from 15 noncancerous volunteers served as controls. None of the subjects had received chemotherapeutic

* Assistant Professor in the Department of General Surgery and Medical and Surgical Research Center, Hacettepe University School of Medicine, Ankara, Turkey.

agents, radiotherapy nor blood transfusions previous to sampling. The test group consisted of 14 blood samples obtained from patients suffering only from gastrointestinal carcinoma. Tissue samples of the test group for histological diagnosis were collected during the abdominal exploration and they were routinely processed and stained with hematoxylin-eosin for microscopic examination. The blood samples were brought to the cold room within a few minutes after venipuncture and all subsequent manupulations were done immediately. The blood, anticoagulated with heparin, was suspended directly in distilled water and the GSH levels of red cells were determined by the method of Beutler, et al.[10]

Results

Table I demonstrates the differences in GSH levels in normal controls and patients with advanced carcinoma of the gastrointestinal tract. The statistical averages of GSH content of the erythrocytes were measured by the method of Beutler et al as 128.34 ± 8.06 mg/100 ml in the test group and 72.71 ± 4.77 mg/100 ml in the control group. The correlation coefficient was highly significant between these two groups ($p < 0.001$). There was no clear correlation between the increasing rate of red cell GSH and the invasiveness or dissemination of cancer tissue found during the abdominal exploration.

TABLE I

GLUTATHIONE LEVELS OF THE RED BLOOD CELLS IN NORMAL CONTROLS AND PATIENTS WITH ADVANCED CARCINOMA OF THE GASTROINTESTINAL TRACT (MG/100 ML)

No.	Control Group	Test Group
1	88.27	122.40
2	86.54	124.30
3	88.41	125.00
4	58.08	159.50
5	64.65	214.20
6	73.50	108.28
7	59.62	105.50
8	64.44	110.00
9	82.89	106.00
10	82.52	153.80
11	78.59	117.00
12	73.81	135.40
13	67.49	114.40
14	35.45	105.00
15	86.52	— —

Discussion

It has been clearly established by several studies that the oxidation of glucose-1-^{14}C to $^{14}CO_2$ is a measure of pentose phosphate pathway (PPP) activity in mature, non-nucleated erythrocytes.[11, 12] Jacob and Jandl have reported that the activity of the PPP of red cells, assayed by $^{14}CO_2$ production from glucose-1-^{14}C, is regulated primarily by GSH.[6] The PPP activity changes of red cells have been observed in a basal or unstimulated state and in the same cells after the stimulation of metabolic activity.[9] Szeinberg and Marks showed that an increase occurred in PPP activity associated with the GSH elevation in erythrocyte following the addition of methylene blue and the other oxidant drugs or at high O_2 concentration.[13] Recently, Davidson and Tanaka demonstrated that increases of systemic temperature above 37°C in the range found in febrile patients, at 39°C and 40.5°C, result in an increase in PPP activity of the unstimulated red cell by 24 % and 41 %, respectively.[9] According to these data, the factors stimulated the PPP activity that exert an oxidative stress on the red blood cell, in particular, and some of these cause haemolysis.

Adelsbreger and Becker demonstrated the red cell of patients with cancer showed significant hemolysis when they were tested with high-speed supernatants which were prepared from human cancer tissue.[14] The ultimate destruction of the erythrocyte is associated with membrane damage. In the mature erythrocyte lacking the apparatus for division or protein synthesis, the major role of GSH has to be the prevention of membrane injury against the oxidative stress.[5, 15] The presence of a specific cytotoxic factor in blood, elaborated by tumor tissue which might damage the red cell membrane was confirmed by some detailed studies.[16, 17]

Most probably, the membrane integrity of erythrocyte is protected by cellular GSH against this oxidative effect of the tumour.

This study suggested that the higher levels of GSH in erythrocyte of humans with advanced gastrointestinal tract carcinoma, indicate an oxidative stress in these cases. Further investigations regarding the nature of this increase in GSH level of the red cell are at present in progress.

Summary

The most important function of glutathione in the mature red blood cells is prevention of membrane injury. The erythrocytes of patients with cancer showed significantly increased glutathione concentrations

relative to the normal human red blood cells. This alteration is most probably related to the oxidative effect of a substance elaborated by tumor tissue. The statistical average of erythrocyte glutathione values were determined by the method of Beutler et al. as 128.34 $\pm$ 8.06 mg/100 ml in the test group and 72.71 $\pm$ 4.77 mg/100 ml in the control group.

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Insulin Transport Across Placenta*

(A Preliminary Report)

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Introduction

After the introduction of insulin to the treatment of diabetes, the possibility of diabetic women becoming pregnant increased and even approached that of normal women. Despite its benefits to the patient, insulin may have undesirable effects on the fetus.[1, 2, 3] Some investigators claim that use of insulin even at therapeutic range may lead to fetal malformations. In order to find out to what extent this opinion is valid, it is necessary to know first how much of the insulin in the maternal blood is transported across the placenta. In literature there are many papers published on this subject; their results, however, are inconsistent. While Gitlin et al.[4] found a 33 % insulin transfer across the placenta, Keller and Krohm[5] doubted if any was transported, while others affirmed that no insulin passed across the placenta.[6, 7, 8, 9]

In the present study, our purpose was to re-examine this question by using insulin labeled with a radioisotope which would yield high specific activity and would enable us to use large doses without any radiation hazard. Such an isotope is ^{99m}Tc which has a physical half-life of 6 hours and relatively low gamma energy ($E\gamma$ = 140 keV). Despite these favorable characteristics, ^{99m}Tc had not been used to label insulin before. In previous studies either ^{131}I ($T_{1/2}$ = 8.08 days) or ^{125}I ($T_{1/2}$ = 60 days) labeled insulin was employed. For comparison we used both ^{125}I and ^{99m}Tc labeled insulin in the present investigation.

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Material and Method

Labeled Insulin: ^{125}I labeled insulin was obtained from Amersham, England. It had a specific activity of 50 μ Ci/μ g and a chemical concentration of 20 nanograms insulin/ml.

^{99m}Tc labeled insulin was prepared in our laboratory. ^{99m}Tc was obtained in the chemical form of $NaTcO_4$. In order to label a molecule with Tc, it is necessary to reduce Tc from + 7 oxidation state to + 4. For this purpose, we used Sn^{+2} which is routinely employed in other radiopharmaceutical preparations.[10, 11]

The following procedure was used to label insulin with ^{99m}Tc: 10 mg $SnCl_2 \cdot 2H_2O$ was dissolved in 10 ml distilled water. The pH was adjusted to 4 with 0.5 N NaOH. 1 ml of this solution was combined with 3 ml (5 mg) insulin solution and the mixture was stirred for 5 minutes. The solution was then filtered through a membrane filter (Millipore, pore size: 0.22μ) and collected in a sterile vial. A sterile solution of Na $^{99m}TcO_4$ containing the desired amount of radioactivity was then added to the above solution while stirring.

The amount of free, unlabeled $^{99m}TcO^-_4$ was checked by descending paper chromatography (85 % methanol). The labeled protein stays at the origin and the free pertechnetate moves with an Rf value of 0.60-0.70. Unbound pertechnetate was always found to be less than 1 % of the total radioactivity.

Guinea Pig Experiments: 25 guinea pigs in the second or third trimester of their pregnancy with a weight average of 750-900 g were anesthetized with a mixture of sodium nembutal (30 mg/kg) and 0.01 0.15 ml Combelen. The animals were fixed on a cork dissection table. The carotid artery was catheterized. At the beginning of each experiment-a blood sample was taken from the mother guinea pig for the blood sugar determinations (Somogyi-Nelson micro method).

After laparotomy the cornu uteri were spread out. 0.1μCi ^{125}I-Insulin in 2 ml solution was injected through the catheter. At 15, 30 and 60 minutes following injection blood samples were taken first from the mother and then from the heart of each fetus. They were kept at - 20°C for 1-2 days until radioactivity counting by means of a Tri-Carb scintillation spectrometer (Packard 2001).

Dog Experiments: 10 pregnant dogs weighing 15-20 kg were prepared for operation. They were anesthetized by the same drugs. V. femoralis was catheterized and after laparatomy cornu uteri were exposed. 0.129 mCi ^{99m}Tc-insulin (4 U.S.P. units) in 1 ml solution was

injected through V. femoralis. Blood samples were taken at 15, 30 and 60 minutes and the radioactivity was measured as outlined above. All the radioactivity counts were corrected for the background.

Results

Guinea Pig Experiments: The radioactivity counts in 1 ml blood of guinea pigs reached a level of 44-236 cpm (mean: 141 ± 43) at 15 min, 66-202 cpm (mean: 118 ± 43) at 30 min and 60-224 cpm (mean: 127 ± 41) at 60 min (Table I).

In 1 ml fetal blood the radioactivity was 0-60 cpm with a mean of 15 ± 12 in 71 fetuses at 15 min, 0-80 cpm with a mean: 17 ± 16 in 66 fetuses at 30 min and 0-95 cpm with a mean: 11 ± 14 in 53 fetuses at 60 min (Table I). The ratio of radioactivity concentration in the blood of fetus to that of mother was 10.6 % at 15 min, 14.4 % at 30 min and 8.7 % at 60 min.

Dog Experiments: The radioctivity was 1764-22800 cmp (mean: 20309 ± 1563) at 15 min, 4201-6098 cpm (mean: 5161 ± 496) at 30 min and 4073-6078 cpm (mean: 4980 ± 508) at 60 min in 1 ml of dog blood (Table I).

TABLE I

RADIOACTIVITY COUNTS IN 1 ML OF BLOOD (CPM)

		Time (min)		
	Animal	15	30	60
Guinea Pig	Mother	141 ± 43	118 ± 43	127 ± 41
	Fetus	15 ± 12	17 ± 16	11 ± 14
	% Radioactivity crossing placenta	10. 6	14.4	8.7
Dog	Mother	20309 ± 1563	5161 ± 496	4980 ± 508
	Fetus	103 ± 21	114 ± 19	94 ± 14
	% Radioactivity crossing placenta	0. 5	2.2	1.9

53 dog fetuses were studied. In 1 ml blood the radioactivity was 75-180 cpm (mean: 103 ± 21) in 49 fetuses at 15 min, 76-180 cpm (mean: 114 ± 19) in 47 fetuses at 30 min and 72-130 cpm (mean: 94 ± 14) in 47 fetuses at 60 min (Table 1). The ratio of radioactivity concentration in the fetus to that of mother was 0.5 % at 15 min, 2.2 % at 30 min and 1.9 % at 60 min.

Discussion

Our results show that some activity was transported across the placenta. This ratio ranged from 11 to 17 % in guinea pigs and 0.5 to 2.2 % in dogs. Guinea pig results, however, are misleading because the activity circulating in the pregnant guinea pig is no more than 140 cpm/ml and it is 17 cpm/ml in the fetus, which is a very small figure for dependable results.

Dog experiments were done with ^{99m}Tc-Insulin. As a result of more radioactivity being administered, the counts were far above the background and the results were more dependable. The activity transported across placenta was less (0.5-2.2 %).

Our results are similar to those of Gitlin et al.[4] However, the activity transported (33 %) in their experiments is higher than ours. They declared that insulin crossed placenta in both ways. Buse et al[12] reported that placenta has relative impermeability. They, however, could not deny slow transport. In this case the passage would most likely be unimportant for the fetus. According to these authors the placenta is not only a barrier to insulin, but at the same time it captures and destroys the hormone.

Turner et al.[13] did not find any correlation between the insulin levels of the maternal and fetal blood. They decided that it was a weak probability that the insulin in the mother's blood should pass the placenta. Adam et al[9] found some insulin fragments in the fetus after administering ^{131}I labeled insulin to the mother, however, they did not know whether insulin was fragmented by the mother's tissue, placenta, or by the fetus.

In another study where radioactive iodine was administered to the mother, iodine concentration in the fetus reached 50 % of that of the mother at 15 min and up to 90 % at 80 min, indicating that unbound iodine crosses placenta very easily.[14]

Although most authors report the presence of some activity in the fetal blood, the question as to which molecule the radioisotope is attached is obscure. The activity circulating in fetal blood might be due to free ^{125}I (I^-) or ^{99m}Tc (TcO_4^-) labeled insulin or labeled framents of insulin. Thus, it is necessary to analyze the radioactivity in the fetal blood by chromatographic methods before definite statements can be made on this subject. More radioacitivity should be administered to the mother to obtain enough activity in fetal blood for the chromatographic analysis. This type of study is only possible with ^{99}Tc labeled insulin.

Summary

The question of whether insulin is transferred across placenta is at present an important unsolved problem which deserves re-evaluation. In our experiments we used two series of animals with "haemochorial" placenta, namely guinea pigs and dogs and insulin labeled with two different radio-isotopes, either ^{125}I or ^{99m}Tc. After administration of the labeled hormone, blood samples were taken from the mother and fetus for radioactivity counting. Our results show transfer of radioactivity across placenta as expressed by the ratio range of 8.7-10.6 % for guinea pigs and 0.5-2.2 % for dogs.

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Binding of the Wedge-Presumed Initiator of DNA Replication-to DNA*

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A few years ago we isolated a low molecular weight factor from Ehrlich ascites fluid of mice and showed that it had the properties expected of an initiator of DNA replication; we have named it "the wedge".[1] This factor was later separated into two fractions[2] and it was demonstrated that thymidine uptake was stimulated by these fractions, in tobacco pith tissues.[3] As two of the properties of the wedge were stimulation of DNA polymerase reaction *in vitro* and enhancement of cell division in protozoa cultures[1] it was of interest to find out the mechanism by which these effects were elicited. We speculated that in analogy to the σ factor of RNA polymerase, the wedge might bind to DNA and make new regions on the template available to the enzyme. This communication deals with the preliminary test of this speculation.

Materials and Methods

Wedge fractions were isolated from Ehrlich ascites fluid of mice, by Sephadex G-25 chromatography as already described,[2] however, 0.1 M ammonium acetate solution (pH: 8.0) was used instead of 0.01 M KCl. During rechromatography of peak II, a partial resolution into 2 fractions was observed.

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Labelled and unlabelled nucleosidetriphosphates and labelled dimethylsulphate were purchased from Schwarz-Mann (Orangeburg, N. J.). Sephadex G-25 was obtained from Pharmacia Fine Chemicals (Piscataway, N.J.). All other chemicals used were of analytical reagent grade. Radioactivity measurements were made with a Tricarb liquid scintillation counter using thixotropic gel.

Experimental and Results

Because of the small size of the wedge the reagent chosen for labelling was one that could react with many groups on the molecule. For this reason it was decided to use labelled dimethylsulphate. To 1 ml of a solution of the wedge fractions (20 mg/ml) in 0.005 M potassium phosphate buffer (pH: 10.0) was added 30 μci(0.3 ml of 100 μci/ml) of dimethylsulphate and the mixture was incubated for 8 hours at 37°C. The reaction mixture was chromatographed on Sephadex G-25 using 0.005 M potassium phosphate buffer (pH: 7.0). The lower half of Figure 1, which represents a typical chromatogram obtained this way, shows that the wedge fraction used was labelled during this procedure. The upper half shows a control experiment whereby a mixture of the same fraction with labelled dimethylsulphate in 0.005 M phosphate buffer (pH: 7.0) was chromatographed on the same column. The pH of the buffer as critical because even at (pH: 8.0) partial demethylation occurs and one obtains two labelled peaks emerging from the column.

The pooled peak *a* (fractions 5-8, 18 ml) and peak *b* (fractions 10-15, 20 ml) were lyophilized and dissolved in 2 ml of distilled water, yielding solutions of the respective fractions in 0.045 M and 0.050 M potassium phophate buffer (pH: 7.0) 0.4 ml of these solutions were mixed with 0.2 ml of either single or double stranded calf thymus DNA solution (0.5 mg/ml), 0.01 ml of $ZnCl_2$ ($1x10^{-5}$M) and 0.01 ml of $MgCl_2$ ($1x10^{-4}$M) and left to stand overnight at room temperature. The mixture was then chromatographed on Sephadex G-25 using 0.005 M potassium phosphate buffer (pH: 7.0) as eluent, Figure 2 demonstrates the results of such a chromatography using material obtained from peak *a*. The upper half of the figure shows the binding to double and lower half shows the binding to single stranded DNA. Results obtained using material from peak *b* are almost identical. There is no interaction between either single or double stranded DNA (Figure 3) and dimethylsulphate under the conditions of these experiments.

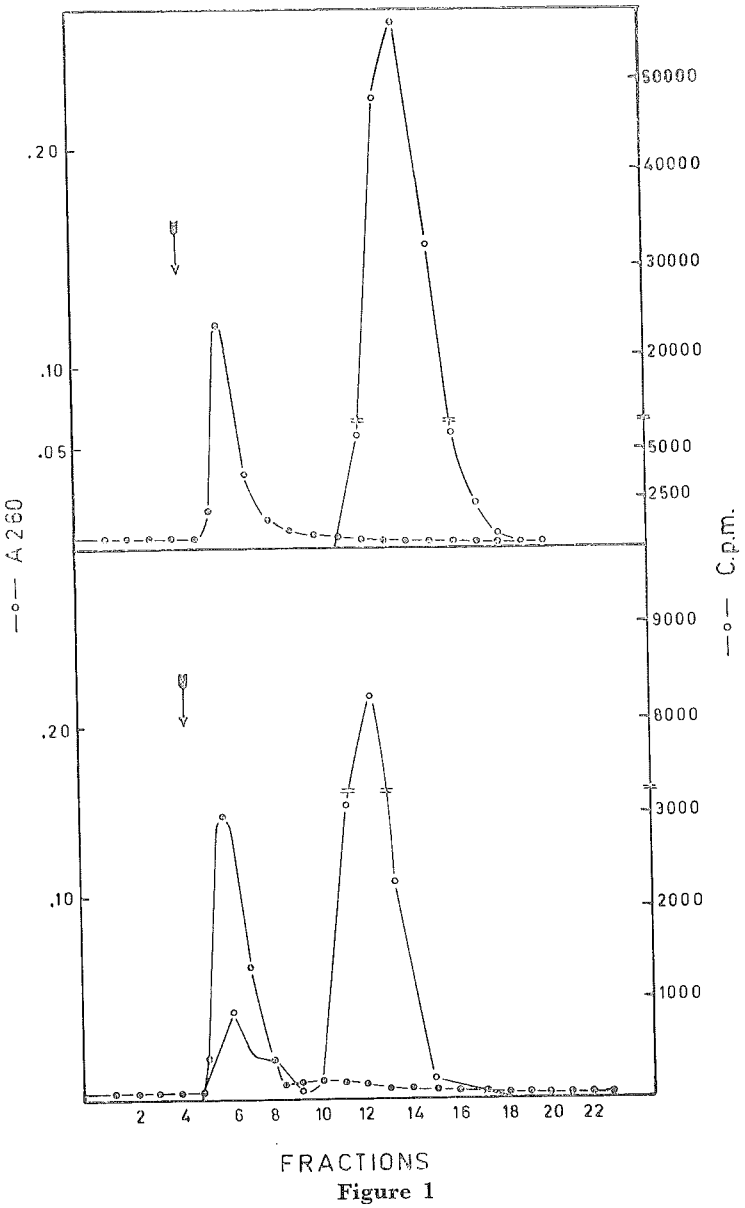

Figure 1

Lower half: Chromatogram of methylation mixture, consisting of tritiated dimethylsulphate and peak II of the wedge in 0.005M potassium phosphate buffer(pH:10.0), incubated for 8 hours at room temperature, on Sephadex G-25 0.005 M potassium phosphate buffer (pH:7.0) was used as the eluent.

Upper half: Chromatogram of the mixture of dimethylsulphate and wedge, incubated in (pH:7.0)buffer and used as control, on the same column.

Arrow indicates the exclusion volume.

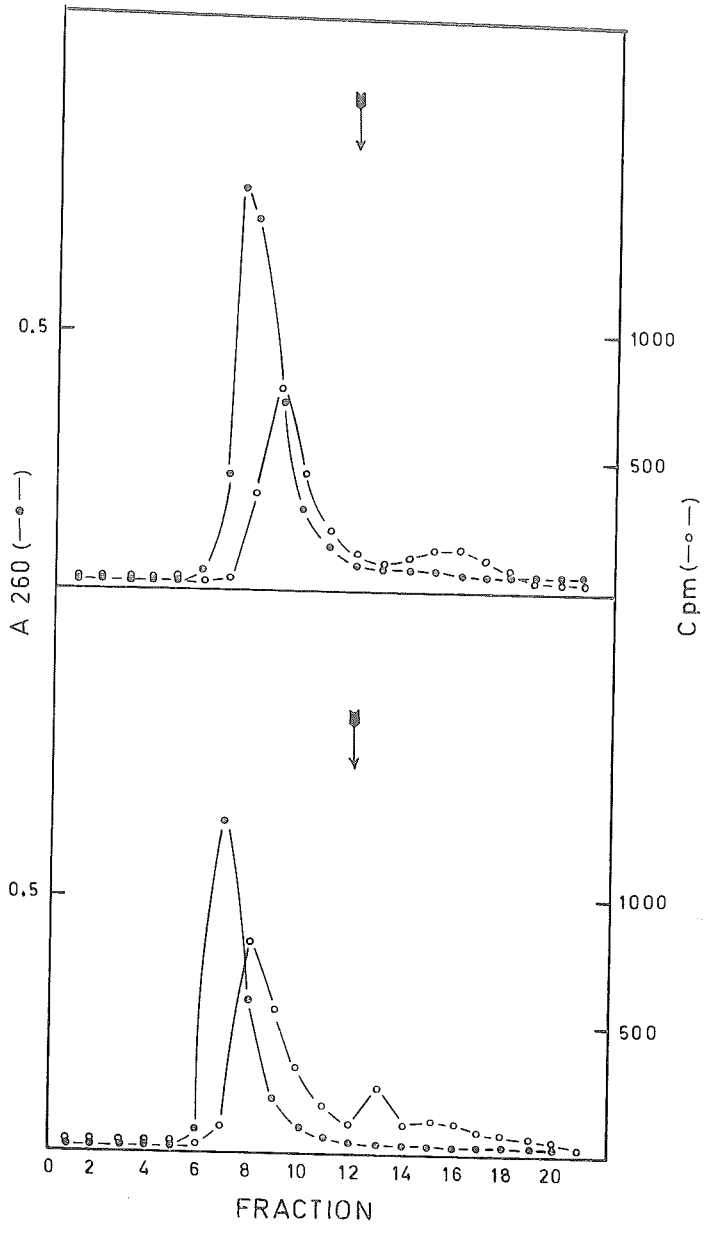

Figure 2

Lower half: Binding of methylated wedge(peak a) to single stranded DNA, determined by Sephadex G-25 chromatography.

Upper half: Binding of the same fraction to double stranded DNA, under identical conditions given above. Arrow indicates the exclusion volume.

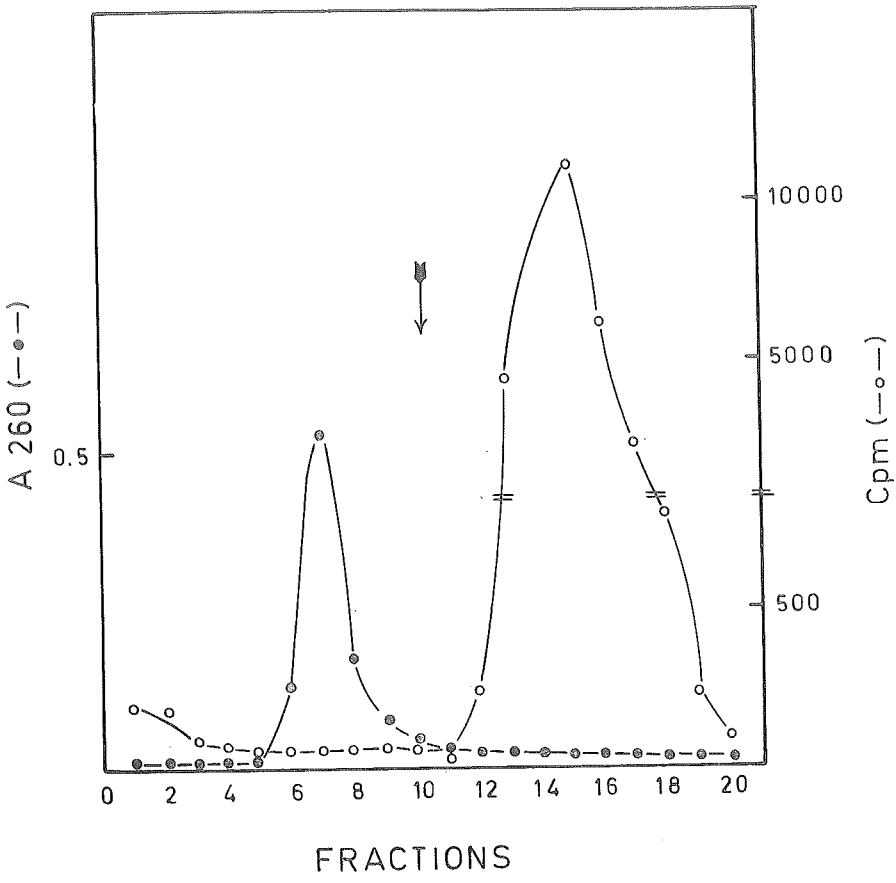

Figure 3

Control experiment demonstrating that dimethylsulphate does not bind to or react with double stranded DNA under conditions used for binding studies, the details of which are given under experimental section.

Approximately 75 % of the material in peak *a* is bound to DNA:
22440 cpm (bound to DNA)

30174 cpm (bound to DNA and unbound)

Since there is 240 μg of material in this peak (600 μg x 0.4 ml = 240 μg), about 180 μg of this was bound to DNA(240 x 0.75 = 180 μg). Assuming a molecular weight of 5000 for this material[2] the bound portion corresponds to ca 36 mμ moles. On the other hand 100 μg DNA corresponds to approximately 300 mμ moles of nucleotides. Hence a stoichiometry of 36 mμ moles of the wedge to 300 mμ moles of DNA nucleotides or roughly 1:8 is obtained.

This binding is resistant to DNase treatment as well as to salt concentration up to 0.2 molar.

Discussion

Stimulation of *in vitro* DNA polymerase reaction by the wedge could be due to three reasons:

1) The wedge might have nuclease activity producing additional 3' -OH ends, which act as primers;
2) The stimulation is a phenomenon dependent upon binding of the wedge to DNA;
3) The stimulation is neither due to the presence of nuclease activity nor to binding of the wedge to DNA, but is because of some yet unexplainable reason.

We already have published the results of some experiments that demonstrated the absence of nuclease activity in early wedge preperations.[1] The results obtained in this study provide an answer to this question by demonstrating a binding of the wedge to DNA. The binding appears to be rather strong, occuring at low ionic strength. During late stages of this study it was possible to show that binding could occur within an hour under the same experimental condition. Resistance to 0.2 M salt concentration brings to mind a possible similarity between the interactions of wedge-DNA and histone-DNA. However, this question is being further investigated. Appearance of 2 peaks during isolation of the labelled fraction II probably reflects the microheterogenous nature of the wedge since this partial resolution appears with regularity during rechromatography.

Recently the isolation of DNA binding protein,[4] T4 induced "gene 32" protein[5] involved in the replication and recombination of the phage, were reported. Both of these proteins were isolated by virtue of their binding to DNA. It was demonstrated that in the presence of "gene 32" protein T4 DNA polymerase was capable of using single-stranded templates much more rapidly.[6] Later it was observed that addition of DNA unwinding protein of *E. coli* could stimulate repair catalyzed by DNA polymerase II.[7] Hence, it appears that these DNA-binding proteins have a preference for single-stranded DNA and the stoichiometry of binding is DNA: protein:=1:12 on a weight bases for "gene 32" protein. The cooperative nature of binding leads to uniform coating of single stranded DNA, with protein in excess, which can be visualized by electron microscopy.[8]

Compared with these, the binding of the wedge to DNA occurs with both single, as well as double stranded DNA, and at a considerably lower level of the factor. Furthermore, the wedge also binds to DNA polymerase.[9] These observations make it a likely candidate for being an initiator of DNA replication.

Summary

A low molecular weight factor, isolated from Ehrlich ascites fluid of mice and shown to possess properties expected of an initiator of DNA replication, was labelled with tritiated dimethylsulphate. The labelled factor is bound to DNA when it is incubated with either single or double-stranded calf thymus DNA.

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Osteoarthritis and Biomechanics of Tibial Osteotomy

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Introduction

The word *arthritis* literally means inflammation of a joint. This word is widely used to cover various conditions which lead to aches and pain in joints and connective tissues in different parts of the body; however, not all of them necessarily involve inflammation. The word "*rheumatism*", used for unexplained aches and pains in joints and muscles, is vaguely defined even by the medical experts in the field. For example, in Great Britain, this word is used to include most forms of arthritis, whereas in the United States the word "*arthritis*" is a widely used term covering rheumatism as well as other conditions. Probably the most significant characteristic of the major forms of arthritis is that it is chronic; i.e., once it starts, it tends to get worse and continues for a lifetime.

This paper is intended to serve two purposes, namely, to be a tutorial paper on osteoarthritis and tibial osteotomy and to be a paper delineating the biomechanics of tibial osteotomy. In the first sections, brief background information on common forms of arthritis will be introduced. This will be followed by a section dealing with osteoarthritis. In the last and the major section of the paper, the tibial osteotomy will be investigated in great detail by means of topics dealing with patient selection, location of osteotomy, determination of wedge size, and operation technique.

Common Forms of Arthritis

Rheumatoid arthritis and osteoarthritis are the most frequently encountered form of arthritis. Rheumatoid arthritis is probably the most serious and most crippling form of arthritis. Although the joints of the

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body are the primary targets for this inflammatory and chronic disease, it can also affect the internal organs such as lungs, heart, and blood vessels, muscles, skin and even the eyes. At the joints inflammation and thickening of the synovial membranes causes irreversible damage to the articular cartilage and to the joint capsule as these structures are replaced by scar tissue. Under the layer of this scar tissue the cartilage is eventually eroded and destroyed. In the late stages deformity and ankylosis develop, thus leading to disuse atrophy of the adjacent bones, muscles, and skin. The chief symptoms of rheumatoid arthritis are usually weakness and fatigue; loss of appetite; local soreness, stiffness and pain in a joint or in several joints; swelling; and heat accompanied by muscle pain that may persist for weeks or subside after a short period. The cause of rheumatoid arthritis is not known. However, there are two leading theories:1) The first one is known as *autoallergy;* i.e., a derangement of the body's own immune mechanism which produces antibodies that attack joints and tissues. The supporting evidence for this theory is the presence of a characteristic group of immunoglobulins (proteins with antibody activity) in the blood of most patients with rheumatoid arthritis. 2) The second theory asserts that rheumatoid arthritis may be caused or triggered by a virus of some kind. Despite the persistent search for such a virus in the affected areas, none has been found yet. Of course, there is always the possibility that the virus may simply disappear after the disease develops. Since each case of rheumatoid arthritis is individual and different from all others, there is no single pattern of treatment that is used for every person with rheumatoid arthritis. Physical therapy and various drugs are used to relieve pain or control inflammation, or both. Orthopaedic surgery on joints is performed to some extent either to check the progress of the disease or to correct gross deformity and malfunction.

Besides rheumatoid arthritis and osteoarthritis which will be discussed in some detail in the next section, there are three more forms of arthritis which are frequently encountered. These are: (i) *Rheumatic fever*, which is an acute disease whose exact cause is not clear, but it is generally thought to result from the reaction of the body to one or more products of the hemolytic streptococcus. It is characterized by fever and inflammation of the joints and heart. Symptoms of cardiac involvement include increased heartbeat rate, heart murmurs, heart enlargement, inflammation of the heart muscles and supporting structure. Other signs of rheumatic fever include: abdominal pain, nosebleeds, weakness, loss of appetite and body weight, nodules beneath the skin, and skin rashes. Generally the clinical symptoms and after-effects of rheumatic fever range from unnoticed conditions to a severe, acute attack associated

with cardiac failure. The arthritis caused by rheumatic fever usually subsides quickly without crippling. (ii) *Ankylosing spondylitis* is a chronic inflammatory arthritis of the spine and larger joints, mainly affecting adolesoent and young adult males in the early twenties. If untreated, it may result in complete ankylosis of the spinal column. Although it is histologically similar to rheumatoid arthritis, most researchers consider it a separate disease. With morning backache as an early symptom, the condition later results in swollen joints, progressive deformity, and anemia. (iii) *Gout*, also called "*gouty arthritis*", is a hereditary metabolic disease characterized by acute attacks of severe inflammation and pain in small joints of the lower extremities, especially the big toe. Gout results from the deposition of salts of uric acid in and about the joints. Redness of the skin, heat, extreme tenderness, and pain of the affected joint are the symptoms of gout.

Osteoarthritis

Osteoarthritis, more properly called degenerative disease of the joints, is a form of chronic arthritis occurring chiefly in aging adults and characterized by the progressive deterioration of articular cartilage and by the reactive response of the dense bone in the form of bony projections at the boundaries of the articular surface. Unlike rheumatoid arthritis, osteoarthritis does not affect the whole body and is a noninflammatory disease of joints, particularly the weight bearing joints such as hips, knees and spine. Although osteoarthritis has other names like arthrosis, osteoarthrosis and hypertrophic arthritis, the most popular and probably the most appropriate name has been "degenerative joint disease". The names osteoarthritis and hypertrophic arthritis are somewhat misleading since the suffix "-itis" automatically implies the existence of inflammation. In osteoarthritis inflammation of joints can occur when the two forms of arthritis; i.e., rheumatoid arthritis and osteoarthritis, exist at a joint at the same time. Fortunately, this "mixed arthritis" is not as common as either one of its components.

Osteoarthritis can be classified as man's oldest and most common disease. As a matter of fact, osteoarthritic changes have been seen in skeletal remains of Neanderthal man and in a large variety of animal species. By some, the deterioration of articular cartilage is considered as an inherent part of the aging process. Hence, every aging person is expected to have osteoarthritis to some degree. Besides being considered as an apparent consequence of the aging process, osteoarthritis is also strongly associated with conditions such as excessive cartilage wear due to high stresses at the joints, abnormal joint mechanics, injuries, and

certain metabolic disorders that affect articular cartilage directly. In early stages of degenerative joint disease there is a softening, pitting and roughening of the smooth cartilage surface. The cartilage looses its normal viscoelastic properties and becomes more susceptible to additional damage due to external stress. As the disease progresses, partial or total destruction of the cartilage takes place and the underlying bone is exposed. In very severe osteoarthritis the normal geometry as well as mechanical function of a joint may be destroyed.

High stress and injury-related osteoarthritis is called "secondary" osteoarthritis because of the fact that the disease is secondary to or the the result of, such biomechanical action on the joint. If no apparent cause or event is known and the degenerative joint disease starts somehow by itself, it is named "primary" osteoarthritis. Primary osteoarthritis affects mostly the small joints of fingers and toes and occurs somewhat earlier in life. In contrast, secondary osteoarthritis occurs late in life, affects the larger weight-bearing joints, and may appear in the small joints if an injury and abnormal stresses are present. In both kinds, the number one symptom is pain which is usually confined to the affected joint and ranging from a mild aching with movement of the joint to a constant, annoying pain which persists even at rest. Incidentally, the magnitude of pain in osteoarthritis is not always related to the magnitude of damage in the joint. The second most common symptom is loss of joint mobility and/or stability if a weight-bearing joint like the knee joint is involved. Loss of joint mobility in most situations results in the weakening of the associated muscles and a decrease in body coordination. The generalized symptoms, i.e., fever, loss of appetite and body weight, and feeling sick are not present in osteoarthritis. According to Kyne,[1] the site of osteoarthritis in at least 50 percent of the cases is the knee. The next section is devoted to the osteotomy, more specifically proximal tibial osteotomy, for compartmental tibiofemoral osteoarthritis at the knee joint.

Proximal Tibial Osteotomy

Osteotomy has indeed a long history. It probably started as early as the beginning of the seventh century. The historical treatment of this subject has been analyzed in great detail by Bick.[2] According to Bick, at about the middle of the nineteenth century, Langenbeck in Berlin introduced the method of subcutaneous osteotomy to orthopedic surgery. He, in fact, has received most of the credit for the early work on osteotomy since he is the one who popularized it as a standard technique. With the beginning of the Listerian era of antisepsis in 1865 and with the establishment of multiple methods to obtain better control of the frag-

ments during the early part of the twentieth century, osteotomy became well-accepted.[3]

According to Wardle,[4] the first tibial and femoral osteotomy was performed in 1928 in England to deal with deformities resulting from childhood rickets. The application of either supracondylar (femoral) or tibial osteotomy to deal with an osteoarthritic knee was first reported by Jackson.[5] At that time he stated that the tibia is the preferred site for the osteotomy since supracondylar osteotomy resulted in greater restriction of knee movement than did tibial osteotomy.

Before we proceed with the recent developments on the correction of varus and valgus deformity of the knee by osteotomy, let us briefly describe the basic concept behind this surgical correction and treatment technique. As was stated earlier, osteoarthritis is a progressive deterioration process of the normal knee joint, and most of the time either the medial side (medial tibial plateau) or the lateral side of the joint is involved. Once the mechanical erosion of articular cartilage starts, it automatically changes the normal femorotibial angle, thus causing an abnormal distribution of forces on the tibial plateaus. Note that under normal conditions the femorotibial angle, which is defined to be the angle formed between the anatomical axes of the femur and tibia, is about 171° as shown in Figure 1 (a). In genu valgum the femur tilts laterally reducing the normal femorotibial angle, Figure 1 (b). while in the genu varum the femur tilts medially, thus causing an increase in the normal angle, Figure 1 (c).

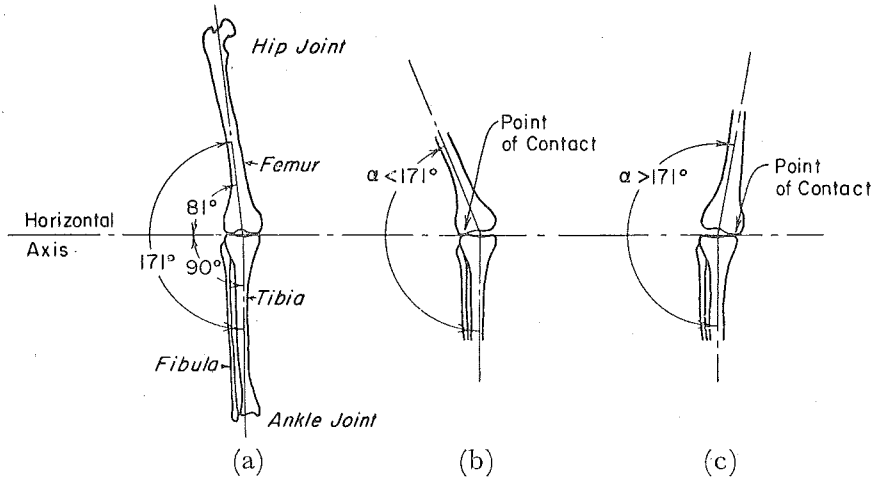

Figure 1
Femorotibial angle for: (a) normal configuration, (b) genu valgum, (c) genu varum.

In previous publications,[6,7] this author and his associates have studied the biomechanics of normal and abnormal (varus or valgus) knee joints. This was done by utilizing a cadaver lower limb which was sectioned at about 25 cm on the femoral and the tibial sides. The skin, subcutaneous tissue, and muscles were dissected away leaving the knee joint capsule intact. Eight strain gages-four on the tibia, four on the femur-were installed at predetermined sites. The bone ends were cast in plaster of paris and connected to the jaws of a specially designed loading apparatus. The experiments were conducted to determine the magnitudes of stress at the strain gaged cross-sections of the femur and tibia for five different configurations; namely, normal, 2.5° and 5° varus and valgus conditions. The abnormal configurations were successively obtained by rotating the tibial end holder of the test apparatus to the desired angle. This rotation of the tibial end in varus or valgus directions resulted in a separation of the lateral or the medial condyles. Analysis of the strain gage data yielded the plateau contact forces for both normal and abnormal conditions of the knee joint. In particular, it was found that the percentage increases in the values of the medial contact force from the normal values are 70 percent for 2.5° varus and 95 percent for 5° varus conditions for the maximum axial test load of 800 Newtons; whereas, the corresponding percentage increases in the lateral force from the normal values are about 50 percent for 2.5° valgus and 75 percent for 5° valgus abnormality for the same test load of 800 Newtons. Thus, for the same degree of varus and valgus abnormality the percentage increase in the magnitude of the contact force is more on the medial side. The affected side's being subjected to higher magnitudes of force than the normal configuration leads to further degeneration of that side and more angular deformity. This is a positive feedback continuous cycle which can be broken down only by mechanically altering the joint geometry to redistribute the forces more favorably for the affected side. The most widely accepted and practical method of redistributing the joint forces is the proximal tibial closing wedge osteotomy which involves removing a suitable size lateral wedge for varus angulation and a medial wedge for valgus angulation (Figures 2 , 3). Before we discuss the indications and contraindications for this surgical procedure, let us examine the highlights of some reported cases and the results obtained. Obviously, the results contain a certain amount of subjectivity and definitions of good, poor or marginal vary from one reporter to the other. Probably the most logical way judging the success of the operation is by evaluating three basic properties such as motion, stability and freedom from pain.[8]

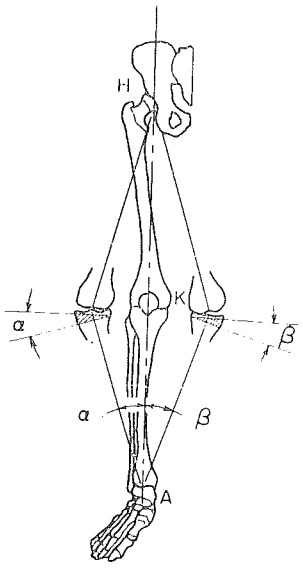

Figure 2

Schematic drawing showing the determination of the wedge angle: α degrees for valgus, β degrees for varus osteotomy, respectively.

Jackson and Waugh[9] reported relief of pain in all eleven patients who had upper tibial osteotomy. They used dome or ball and socket type osteotomy located at or distal to tibial tubercle. This involves first osteotomizing the fibula over its middle third and later convex (either upward or downward) osteotomizing the tibia by a series of drill holes and completing the cut with a gouge. Of thirty osteotomies in twenty five patients, about 73 percent good results were obtained by Venemas.[10] Wardle[11] reported on a series of tibial osteotomies performed no less than 5 cm distal to the tibial tubercle with osteotomizing of the fibula at the same level. Complete relief of pain and at least 90 degrees active flexion were obtained in all but three of seventeen patients. Eighty percent of the results were good as reported by Gariepy[12] in thirteen patients. Coventry[13] presented encouraging results from thirty knees of twenty two patients who had upper tibial wedge osteotomies. Devas[14] categorized his results of twenty-seven supratubercular wedge-type osteotomies as 22 good, 3 fair, and 2 bad. Also, in 1969 Jackson and Waugh[9] reported their further experiences with seventy (46 varus, 24 valgus) osteotomies in which curved osteotomies with upward convexity at the location of the tibial tubercle were performed. They classified their results as 50 knees completely relieved of pain, 17 knees with partial

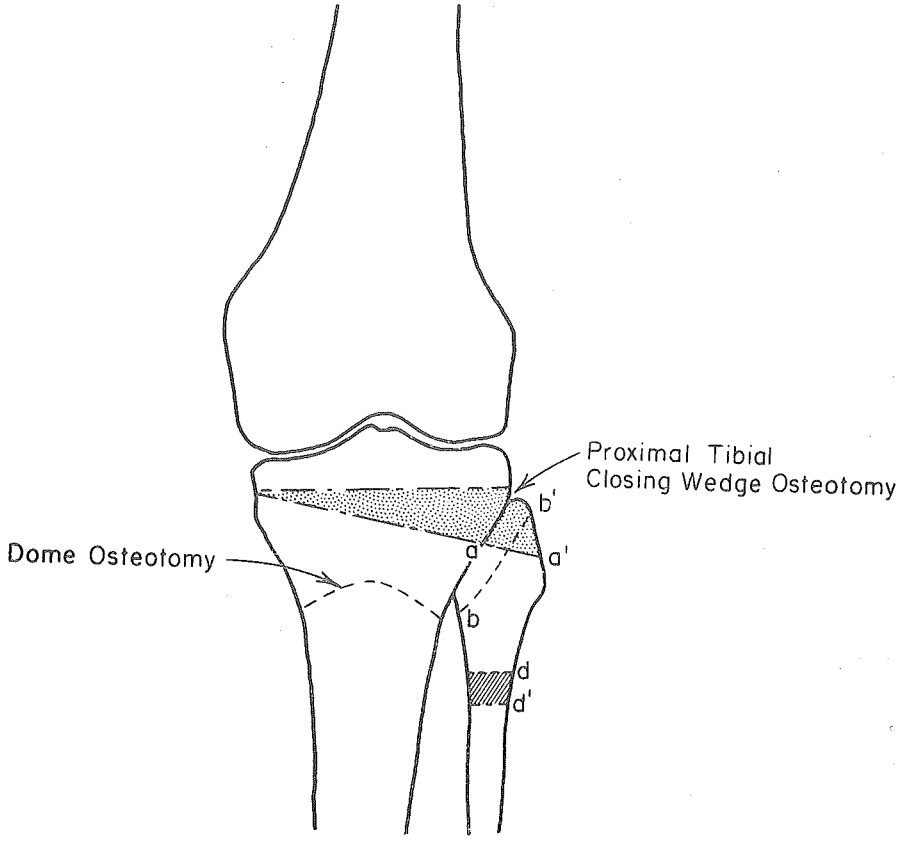

Figure 3

Proximal tibial closing wedge osteotomy and dome or ball and socket type osteotomies are illustrated. Note that a lateral wedge for varus angulation is removed between tibial tubercle and tibial plateaus. Proximal tibiofibular syndesmosis can be handled by: (1) partial resection of head of fibula along aa' or along bb'; (2) release of the joint. Dome osteotomy is located at or distal to tibial tubercle and fibula is osteotomized (dd') over its middle third.

relief, and 3 knees with no relief of pain. Again, in 1969 Bauer et al.[8] documented the results of sixty-six tibial wedge osteotomies in sixty-one patients in terms of freedom from pain and improvement in function and stability. The authors also illustrated the effect of osteotomy by using photoelastic models of the proximal tibia and distal femur to demonstrate the redistribution of stresses to a more normal pattern by osteotomy.

In 1970 Harris et al.[15] reported their experience with forty-five high tibial closing wedge osteotomies performed on thirty-nine patients. Of 36 knees with a year or more follow-up after operation, 26 had good

results, 5 had fair, and 5 had poor results. They used a slightly modified version of Gariepy's technique, which involves staples as advocated by Coventry[13] instead of transfixing pins used by Gariepy for the internal fixation. Later in the year Kyne[1] presented his results of seventeen proximal tibial osteotomies (11 dome-type and 6 wedge-type) in sixteen patients. Pain was fully relieved in 13 knees, in 2 knees reduced, and in the remaining 2 knees no improvement was seen. The complications of tibial osteotomy in children for genu varum or valgum is discussed in some detail by Steel et al.[16] According to these authors, the osteotomy in children must be performed lower in metaphysis than in adults to avoid damaging the proximal tibial epiphysis, and acute arterial ischemia can be a factor in the postoperative phase of the operation. In 1973, Shea[17] reported his results on twelve osteotomies in twelve patients and emphasized that complications and poor results can occur if care is not given in selection of the patients and less than meticulous surgical technique applied. All six patients who were classified in the poor result group showed evidence of arthrosis on both sides of the knee, were obese, and had previous operations. Schweigel[18] published the results of tibial osteotomy on thirty-six knees as two-thirds of the patients had definite relief of pain, and 90 percent had less pain or an improvement of function. Finally, Torgerson et al.[19] reported on their nine years of experience with fortynine patients who had fifty-seven operations with 80 percent satisfactory results based on the evaluation of lack of pain, and good range of motion.

Patient Selection

The available literature on this topic is unfortunately not always consistent. Improper patient selection for the tibial osteotomy may constitute one of the major reasons for poor results and complications. The list given below is basically prepared from references 17 and 19 showing indications and some contraindications for the tibial osteotomy:

1. Osteoarthritis of one tibiofemoral compartment with preferably varus or valgus angularity. The other compartment must be in good shape.
2. No evidence of internal derangement: If an internal derangement from a muscle tear, osteophytes, or any other mechanical obstacle is present and preventing the smooth operation of the knee, intra-articular surgery must be performed first.[20]
3. Stable collateral and cruciate ligaments.
4. At least eighty degrees of motion and less than fifteen degrees of flexion contracture.

5. No more than fifteen degrees of varus or valgus angularity. If the deformity is much more than fifteen degrees, then combination of wedge and dome osteotomy may be considered.
6. Presence of disabling pain and negative results from more conservative modes of treatment.
7. Presence of patellofemoral arthritis is not a contraindication for this operation.
8. Presence of rheumatoid arthritis is a contraindication to tibial osteotomy.

Location of Osteotomy

As mentioned earlier, the tibia has become the preferred site,[8, 9] and the wedge osteotomy is performed above the level of the tibial tubercle. Although the osteotomies were also performed distal to the attachment of the patellar tendon at the tibial tubercle,[9, 10, 11] the recent trend is to perform it above the tibial tubercle and since the bone in this region is cancellous, fast healing is assured, and the healing process is further assisted by the pressure exerted across the osteotomy line by the quadriceps mechanism. Of course, the only disadvantage of the proximal operation is the limited amount of space of availability. This puts an upper bound on the wedge angle, and in certain cases either undercorrections or fracture of the proximal fragment may occur.[8]

Determination of Wedge Size

There is some agreement in the literature that undercorrection usually causes marginal or poor results; however, a lot of controversy exists over whether overcorrection is good or bad. For example, contrary to Bauer et al,[8] Schweigel[18] advocates overcorrection. Of course overcorrection leads to a much higher force distribution on the unaffected side and proportionately reduces the force burden of the affected side. Thus, if error is going to be made, let it be toward overcorrection rather than undercorrection. The wedge angle can be determined by the following simple method. Standing, i.e. in weight-bearing position, a long (from the ankle to the hip) anteroposterior roentgenogram is taken. The standing position is particularly important if the collateral ligaments are weak. There might be easily ten to thirteen degrees of difference for the femorotibial angles corresponding to the supine and standing positions. As is shown in Figure 2, normally, the mechanical axis of the lower extremity (i.e., the line joining the center of the femoral head with the center of the ankle joint, which lies halfway between the lateral and

medial malleous) passes through the center of the knee or midway between the sides of the patella. In the varus deformity an angel, α, between the anatomical axis of the femur and the mechanical axis of the lower extremity is formed. This angle is measured and the value obtained is used for the valgus osteotomy to correct the varus deformity. Similarly, the angle β is used for determining the wedge angle for the varus osteotomy to correct the valgus deformity. Another method of determining the correct wedge angle was suggested by Kettelkamp and Chao[21] and it involves static analysis of the knee joint in the frontal plane. Figure 10 of their work can also be utilized to obtain an estimate of the correction angle.

Operation Technique

Most of the surgeons are using a modified version of Gariepy's technique. Publications by Coventry,[13] Bauer et al.[8] Harris and Kostuik,[15] Torgerson et al.[19] are excellent articles to consult on the details of the operation. A couple of points need to be mentioned here. The first one relates to the nominal thickness of the proximal fragment and the other one to the fixation method. The upper osteotomy cut should be parallel to the joint and should leave at least a two-centimeter-thick proximal fragment. Although transfixing pins, compression devices, and staples have been used by various surgeons, in a normal situation fixation can be achieved without these devices when the knee is kept in a toe-to-groin or cylinder cast for six weeks.[8] Tibial osteotomy can be quite beneficial even if there is not an appreciable amount of valgus or varus deformity. For example, it can be beneficial for a knee joint displaying deterioration of the articular cartilage down the subchondral bone in one side and the normal healthy conditions in the other side.

Summary

Arthritis, more specifically, osteoarthritis and tibial osteotomy were examined via an up-to-date literature review. The pertinent guidelines were presented on proximal tibial osteotomy with topics dealing with patient selection, location of osteotomy, and determination of osteotomized wedge size.

Acknowledgement

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Inhibition by Cycloheximide of Protein, RNA and Cytochrome Oxidase Synthesis in Mycotypha Africana

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Introduction

It is known that cycloheximide (actidone) inhibits the protein and RNA synthesis in mammalian cells and various fungi, except bacteria.[1-5] A dimorphic fungus, Mycotypha africana, grows as yeast-like cells under N_2 and reproduces by budding, and as mycelium in the air.[6,7] The effect of cycloheximide on the synthesis of protein, RNA and cytochrome oxidase was studied in Mucor rouxii, another dimorphic fungus, by Heidle and Storck.[8] These workers showed that the cyloheximide inhibited the synthesis of protein, RNA and cytochrome c during the conversion from yeast to mycelium. It was suggested that this morphological differentiation either in Mucor or Mycotypha was accompanied by the synthesis of protein, RNA and cytochrome oxidase.

In the present paper, the effect of cycloheximide on the synthesis of RNA, protein and cytochrome oxidase was studied in Mycotypha africana during yeast mycelium conversion.

Materials and Methods

Organism

Mycotypha africana (CBS 122-64) was obtained from the Department of Applied Biology, Chelsea College, London. Slopes were grown on Sabauroud-Maltose agar at 30°C for 48 hours and kept at 4°C and were used up to 6 weeks old.

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Media and Chemicals

The medium (YPG) of Bartnicki-Garcia and Nickerson[9] was used. The pH of this medium was adjusted to 4.5 with 1 N H_2SO_4 and sterilized at 121°C for 15 min. Cycloheximide (Sigma) disolved in distilled water and sterilized by membrane filtration (Millipore Ltd. Type HAWP, 0.45 μm).[14] C-Isoleucine and ^{3}H-Uridine was purchased from Amersham Radiochemicals, England, and diluted with distilled water. Cytochrome c from horse heart type III (Sigma) reduced with sodium ditionite.

Inoculation and growth

Spores were suspended with 10 ml of sterilized distilled water and counted in a haemocytometer. The inoculum was adjusted to give 40000 spores/ml of culture broth, and incubated under N_2 at 30° C and 150 rev/min in a metabolite water bath shaker (New Brunswick) with a circular throw of 1.27 cm diameter. At the end of a 42-hour incubation period, labelled chemicals and cycloheximide were added to the culture and exposed to the air, and samples were taken at O min. Cycloheximide was added to the cultures, grown under N_2 for 42 hours and exposed to the air in various cencentrations from 0.1 to 200 μg/per ml of medium. Samples were taken at 0 min of the conversion and in 1 hour intervals and examined under the microscope.

Effect of Cycloheximide on Protein Synthesis

To show the effect of cycloheximide on protein synthesis during conversion from yeast to mycelium ^{14}C-Isoleucine (0.75 μc/ml of culture broth) and drug in the concentration of 1 and 100 μg added to the medium at the 0 min of the conversion. To 2 ml of the samples taken at various time intervals was added 2 ml of cold 10 % TCA (Trichloracetic acid), which was allowed to stand in an ice bath for 15 min. It was then filtered on the millipore filter and washed 6 times with 5 ml of cold 5 % TCA. Dryed filters were mixed with scintillation solution (0.4 % PPO and 0.01 % POPOP in toluen) and counted in a Tri-Carp Liquid Scintillation Spectrometer (Packard Model 3380).

Effect of Cycloheximide on RNA Synthesis

To determine the RNA synthesis ^{3}H-Uridine (3 μc/ml of culture broth) was added to the medium at the 0 min of the conversion. Samples taken in various time intervals were prepared for counting by the same technique as described above.

Effect of Cycloheximide on the Synthesis of Cytochrome c

Cultures grown under N_2 for 42 hours were exposed to the air, and 65 ml samples were taken at 0,240 and 480 minutes and washed by filtration on millipore with two volumes of cold distilled water and then two volumes of 0.1 M phosphate buffer, pH 7.0. Cells were scraped from the filter and homogenized with equal volume of alumina in a mortar. Broken cells were suspended with 4 ml of 0.1 M phosphate buffer (pH 7.0) containing 100 µg cycloheximide per ml. After centrifugation at 4200 rpm for 10 minutes, supernatants were used as enzyme solutions The cytochrome oxidase activity was tested according to the method of Smith.[10]

Determination of protein

Protein was determined by the method of Lowry et al.[11]

Dry weight measurement

To measure the dry weight, 8 ml of the sample was taken at 0 min and 6 hours after exposure to air and filtered on Whatman paper and dried in the oven at 80°C for 18 to 24 hours.

Turbidity measurement

To measure the turbidity a 0.5 ml of sample was diluted with 5 ml of distilled water and read at 450 nm in a Baush-Lomb spectrophotometer.

Results

The Effect of Cycloheximide on the Conversion from Yeast to Mycelium

Germ tube production was not observed in the culture which contained more than 10 µg cycloheximide per ml of medium up to 7 hours after exposure to the air. The time for the appearance of germ tubes in the cultures which contained 5,1, and less than 0.5 µg cycloheximide per ml of medium, were 5, 3, and 2 hours, respectively, after exposure to the air. The delay of the germ tube pruduction was paralleled with turbidity and the dry weight of the culture (Table I).

Effect of Cycloheximide on the Synthesis of protein, RNA and Cytochrome Oxidase

It was found that the protein synthesis inhibited 100 and 92 per cent in the culture which contained 100 and 1 µg cycloheximide per ml

TABLE I

THE EFFECT OF CYCLOHEXIMIDE ON THE YEAST - MYCELIUM CONVERSION. CONTROL CULTURE WAS GROWN UNDER N_2 WITHOUT CYCLOHEXIMIDE. TURBIDITY WAS READ OUT AT 450 nm.

		Cycloheximide µg/ml of medium				
	Control	2	1	0.5	0.2	0.1
OD differences at 0 and 6 hrs	0.015	0.025	0.040	0.050	0.060	0.080
Dry weight(mg) differences at 0 and 6 hrs	2.05	2.19	2.57	5.30	5.24	5.81

of medium, respectively (Figure 1). Inhibition of RNA synthesis was 42 and 40 percent in the concentrations of 100 and 1 µg cycloheximide/ml of medium, respectively (Figure 2). Cytochrome oxidase was also inhibited by cycloheximide during conversion from yeast to mycelium (Figure 3).

Discussion

In the present paper in order to show the relation of yeast -mycelium conversion to protein, RNA and cytochrome oxidase synthesis, cycloheximide was used in Mycotypha africana. It is generally accepted that cycloheximide inhibits protein synthesis in various groups of cells, with the exception of bacteria. Inhibition of RNA synthesis by cycloheximide is not certain. The incorporation of ^{3}H-Uridine in the presence of cycloheximide was not affected in the Disctyostelium discoideum.[5] but RNA synthesis was stimulated by cycloheximide in Saccharomyces pastorianus.[12] Inhibition by cycloheximide of RNA synthesis in Neurospora crassa was related to ribosomal RNA; and in the presence of antibiotic, the methyllation of rRNA could not occur.[1] Fukuhara[2] demonstrated that the induction of respiratory enzymes in the presence of cylohexiimide in Saccharomyces cerevisiae passed to the aerobic growth condition from unaerobic. He found that the inhibition by cycloheximide of portein, RNA and cytochrome c synthesis was 95, 30 and 100 per cent, respectively.

In Mycotypha africana conversion of the yeast to the mycelial form was prevented in the presence of cycloheximide. The cycloheximide concentration (100 µg/ml of culture) inhibited 100 % of the cytochrome oxidase, 100 % of the protein synthesis and 42 % of the RNA synthesis. In this concentration of cycloheximide yeast-mycelium conversion could

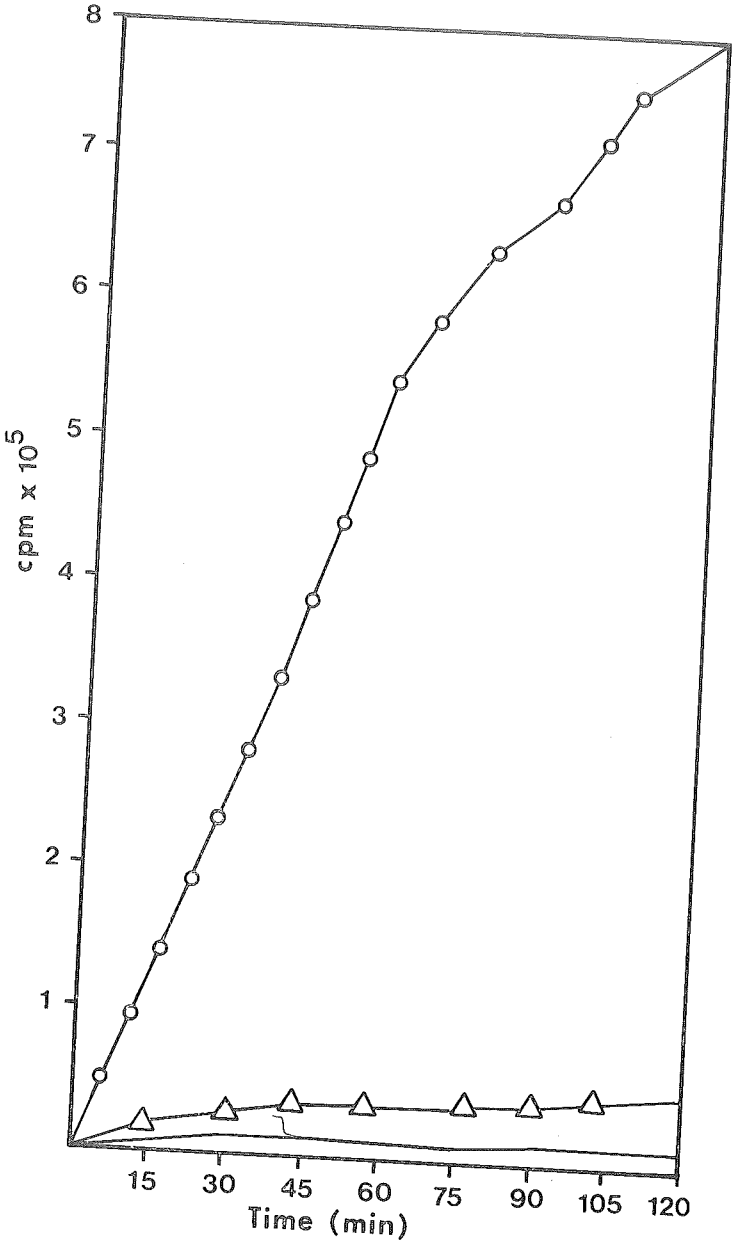

Figure 1

The incorporation of ^{14}C-Isoleucine, with and without cycloheximide: Exposing to the air and labelling (0.75 μc/ml of culture) was carried out at 0 min.

Symbols: o—o, control, without cycloheximide.;Δ—Δ,1 μg/ml cycloheximide was used;—, 100 μg cycloheximide was used per ml of culture medium.

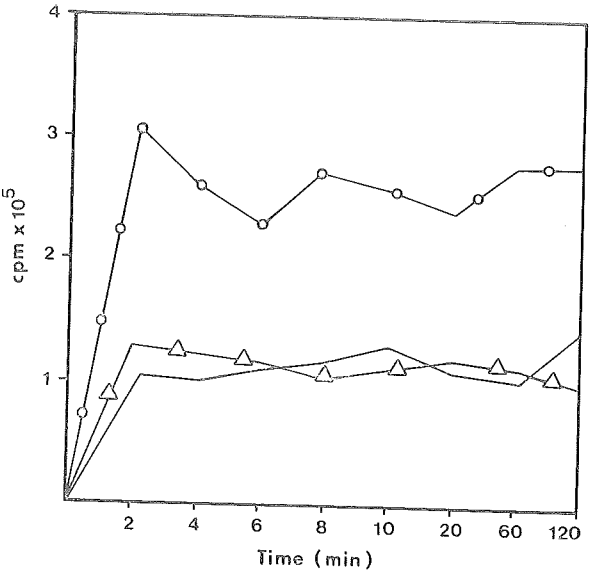

Figure 2

The incorporation of ^{3}H-Uridine, with or without cycloheximide: Cultures were exposed to the air and tracer(3 μc/ml of culture broth) was added at 0 min. *Symbols :* o—o, control, without cycloheximide., Δ—Δ,1 μg cycloheximide was used per ml of culture., — , 100 μg cycloheximide was used per ml of culture.

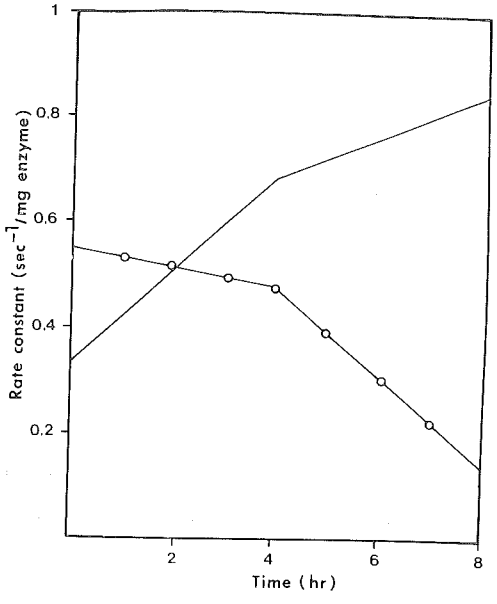

Figure 3

Inhibition by cycloheximide of cytochrome oxidase synthesis during yeast mycelium conversion; —,control; o—o ,100 μg cycloheximide was used.

not occur. When cycloheximide was used in the concentrations of 100 and 1 µg, differences of the per cent inhibition was 8 in the protein synthesis and 2 in the RNA synthesis.

We suggest that these three systems are necessary for conversion from yeast to mycelium. There should be a certain relation between cellular dimorphism and cellular respiration in Mycotypha africana. Therefore, metabolic pathways of the yeast form should be different from that of elial form.

Summary

The effect of cycloheximide (actidone) was studied on the synthesis of protein, RNA and cytochrome oxidase during yeast mycelium conversion in Mycotypha africana. Germ tubes did not produce in the culture grown under N_2 with cycloheximide in the concentration of more than 10 µg/ml of medium, up to 7 hours after passed to the aerobic growth condition. Cultures were grown in the presence of cycloheximide in the concentrations of 5,1, and 0.1 µg/ml of medium germ tubes, appearing by 5,3 and 2 hours, respectively, after passing into the air. It was found that a 1 µg per ml concentration of cycloheximide inhibited 92 % of the protein synthesis, 40 % of the RNA synthesis and 100 % of cytochrome oxidase synthesis.

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