there are marked and constant differences in the quantities of materials excreted–differences which may be as great as 1500%. These workers have correlated certain individual metabolic patterns, particularly those involving vitamin deficiencies, with certain diseases and have shown that these may be successfully treated by dietary supplementation, i.e., they are genetotrophic. They report the extremely encouraging conclusion that even though a physiological condition rests upon hereditary roots, a nutritional attack may be successful in modifying or alleviating it.

Utilizing this concept, we have determined the quantities of more than twenty compounds present in the urine of tumor-susceptible and -resistant rats. Daily and long range excretion variation has been compensated for by pooling five 24-hour urine specimens per individual rat, and repeating the procedure a second time two months later. Quantitative determinations in replicate were made upon each of these two pooled individual samples. Values obtained were corrected for the weight of individual rats so as to make comparisons possible. The number of grams of feed required to be consumed per rat to yeld an excretion of one milligram of a given substance was determined. The Table presents typical data for a single substance (leucine), and serves to illustrate the method employed.

Utilizing the median value for each compound excreted, animals may be ranged according to whether they excrete more or less than this "normal" value. Reported in this manner, it is possible to differentiate most of the susceptible animals by utilizing singly any one of several compounds. These separations are statistically significant, but in each case one or two animals demonstrate aberrant behavior, such that it is impossible to use the results of a single determination. The difference becomes much more striking, however, when several compounds are considered simultaneously.

J. B. LOEFER and R. B. MEFFERD Jr.[1]

From the Southwest Foundation for Research and Education, and Trinity University, San Antonio, Texas, U.S.A., November 18, 1952.

Evaluation of leucine excretion as a means of differentiating between tumor-resistant and tumor-susceptible rats.

Weight in grams*	Milligrams leucine excreted per rat per day	Grams feed consumed per day	Corrected leucine excreted per day**
Susceptible rats			
322	0·26	25·7	88·6
401	0·29	28·3	108·8
351	0·24	26·3	105·2
497	0·33	35·4	147·5
368	0·23	25·1	109·1
450	0·26	25·7	121·4
329	0·24	26·6	98·5
			$\bar{x}_1 = 111{\cdot}3$
Resistant rats			
305	0·27	23·6	73·8
369	0·26	29·2	112·3
405	0·28	27·6	110·4
378	0·29	25·2	90·0
335	0·29	24·1	75·3
270	0·26	17·6	50·3
340	0·25	27·4	101·5
406	0·32	28·1	96·9
280	0·27	23·6	67·4
357	0·33	25·8	75·9
364	0·29	25·8	89·0
			$\bar{x}_2 = 85{\cdot}7$

* Median weight: 364 (weighted to equalize classes).
** Grams feed consumed per day per individual

(milligrams leucine excreted per day per rat) $\left(\frac{\text{median weight}}{\text{individual weight}}\right)$

$x_1 - x_2 = 25{\cdot}6$; $n_1 = 7$, $n_2 = 11$; d. f. = 16; $Sx^2 = 6387{\cdot}7$; $t = 2{\cdot}66$, $P < 0{\cdot}02$; significant at $P < 0{\cdot}03$ level by median test[1].

[1] A. M. MOOD, *Introduction to Theory of Statistics* (McGraw-Hill, New York, 1950), pp. 394.

Zusammenfassung

Es wurde ein Fibrosarkom der Ratte transplantiert, welches sich nicht in allen Tieren der Sherman-Rasse einpflanzen lässt. Über zwanzig Komponenten des Urins von sowohl resistenten wie auch von empfänglichen Tieren wurden analysiert. Die statistische Behandlung der Resultate zeigte, dass bei Tieren aus gleicher Zucht deutliche quantitative Unterschiede in den Urinanalysen existieren. Die Leuzinausscheidung in einem Versuch mit 18 männlichen Ratten übertraf bei den empfänglichen Tieren um 30% diejenige der resistenten von demselben Alter ($P < 0{,}02$). Auch die Ausscheidung anderer Komponenten ist bei den Empfänglichen verschieden von der resistenter Tiere.

[1] Post-Doctoral Fellow in Cancer Research of the Damon Runyon Memorial Fund.

Mammalian Specificity of Fibrinogen

There is no uniformity in the results of investigations concerning the antigen properties of fibrinogens. In some papers[1] considerable differences were reported between fibrinogens of different animals, in others[2] a limited relationship was demonstrated between the fibrinogens of some mammals, while in a third group of papers[3] the relationship is stated to be rather remote. It seems important to clarify this problem both from a theoretical and from a practical point of view. By the fractionation of human plasma different derivatives of fibrinogen are prepared which are successfully used in therapy in different lines of medical practice. The extensive use of these products, however, could not be realized, owing to the fact that they are produced from *human* plasma, a raw material available only in limited amounts, and–in spite of voluntary donors–very expensive. In addition the fibrinogen level in human plasma is very low, and a continous production of the therapeutic derivatives cannot easily be organized. Hence, different substitutes are used such, as gelatina-foam, oxidized cellulose, polyethylen, etc., which are unable to compensate natural proteins in the organism. Since all fibrin products are derivates of fibrinogen, first of all the antigen properties of fibrinogen were studied.

Materials and methods

Preparation of fibrinogen from mammalian and fowl plasma. Fibrinogen was prepared by LAKI's method[4].

[1] T. ASTRUP and S. DARLING, Acta Physiol. Scand. *3*, 311 (1942); *4*, 45 (1942).

[2] H. B. KENTON, J. Immunol. *25*, 461 (1933).

[3] K. KATO, Mitt. med. Ges. Tokio *36* (1922); Ref. Zbl. Bakt. Ref. *75*, 353 (1924). – L. KESZTYÜS, T. SZILÁGYI, I. NIKODÉMUSZ, and T. JÁVOR, Acta Physiol. Hung. *1*, 100 (1950).

[4] K. LAKI, Z. Physiol. Chem. *273*, 95 (1942).

Table I
Precipitin reactions of fibrinogen antisera with homologous and heterologous fibrinogens.

Fibrinogen-antiserum	Antigens: Fibrinogen					
	Beef	Swine	Horse	Sheep	Human	Fowl
Beef	1:1000+	1:500 +	1:250 +	1:250 +	1:500 +	–
Swine	1:1000+	1:2000+ +	1:250 +	1:1000+	1:1000+	–
Horse	1:1000+	1:500 +	1:2000+	1:500 +	1:250 +	–
Sheep	1:250 +	1:500 +	1:250 +	1:2000+ +	1:2000+	–
Human	1:500 +	1:250 +	1:250 +	1:250 +	1:2000+	–
Fowl	–	–	–	–	–	1:150 +

The principle of the method is the following: after repeated fractionating of the plasma with ammonium sulphate, fibrinogen is adsorbed to a gel of tricalciumphosphate, and then eluated in a phosphate buffer. Crystallization of fibrinogen is induced by properly diluting the eluate. Then the crystalline sediment is dissolved in a slightly alkaline (pH 7·5) sodium chloride solution, and it is made free from phosphate and ammonia residues by dialysis against 2% NaCl. Finally fibrinogen is dried from the frozen state. The method was worked out for preparation of swine fibrinogen and it was applied in our experiments in the preparation of fibrinogen of beef, swine, horse, sheep and man on the one hand, and fowl on the other hand.

Estimation of the purity of fibrinogen solution. On the day of the experiment 0·20 g of the dried, lyophilized powders were dissolved in distilled water and the solutions were filtered using SCHLEICHER-SCHÜLL filter paper No. 189. The total protein nitrogen was estimated by micro-Kjeldahl method, and the fibrinogen-nitrogen after clotting (after complete transformation of fibrinogen into fibrin) as fibrin-nitrogen, providing a possibility to remove molecules other than fibrin easily from the meshes of the fibrin-net[1]. The purity of fibrinogens (clottable protein-N in % of the total protein-N) used in our experiments amounts to 96·8% in the beef, 98% in the swine, 91% in the horse, 90% in the sheep, man and in fowl. The concentrations of the fibrinogen solutions used in the experiments were: beef 4·67 mg/ml, swine 4·18 mg/ml, horse 3·23 mg/ml, sheep 5·9 mg/ml, man 4·0 mg/ml, fowl 2·3 mg/ml.

Immunization and anaphylaxis experiments. With each of the six aforementioned fibrinogens 3–3 rabbits of 3000–3500 g weight were immunized. Every third day the animals were injected with increasing dosages of the fibrinogen solutions, altogether with 6 injections, containing totally 70–80 mg fibrinogen. Immunization was well tolerated by the animals, without any loss of weight. Seven days after the last immunizing injection, blood was taken. The precipitin tests were carried out by the layer method, reading after one hour at room temperature. Anaphylaxis experiments were carried out with 60 pigeons, using GAHRINGER's method[2] and with guinea pigs. The pigeons were given intravenously 1 ml fibrinogen solution; after a fortnight "reinjection" of fibrinogen was given again intravenously (1 ml). Anaphylactic symptoms were manifested within 1–4 min, consisting of lacrimation, mucuous discharge from mouth and nostrils, sneezing, dyspnoea (+), and muscular debility (++). The symptoms subsided within 15–60 min; in two pigeons, however, lethal anaphylaxis was observed (+++). Guinea pigs were sensibilized with fowl fibrinogen also; at first 0·1 ml were given subcutaneously, and 25 days later as reinjections of 0·25 ml intracardially.

Absorption tests. In these experiments the antiserum was mixed with an equal volume of fibrinogen solution (in controls with an equal volume of physiological sodium chloride solution or serum). Incubation was performed for one hour at room temperature with occasional shaking of the mixture. The precipitated sediment was centrifuged and the supernatant liquid was used to examine the presence of antibodies.

Results and Discussion

Results of principitin tests carried out with homologous and heterologous fibrinogens are summarized in Table I. *Mammalian fibrinogen antisera precipitate heterologous mammalian fibrinogens also but fail to precipitate fowl fibrinogen. On the other hand, fowl fibrinogen antiserum fails to precipitate any of the mammalian fibrinogens.*

In Table II results of anaphylaxis experiments are demonstrated. *In pigeons sensitized with any of the mammalian fibrinogens anaphylaxis was excited by all of the mammalian fibrinogens examined.* On the other hand no anaphylaxis was caused, when the pigeons were given fowl fibrinogen as "reinjection". We were unable to sensibilize pigeons with fowl fibrinogen. *In guinea pigs sensitized with fowl fibrinogen, anaphylaxis was excited by* reinjection of *fowl fibrinogen only*, and *not by any of the mammalian ones.*

Table II
Anaphylaxis reactions* of sensibilized pigeons with homologous and heterologous fibrinogens.

Reinjection	Sensibilisation: Fibrinogen					
	Beef	Swine	Horse	Sheep	Human	Fowl
Beef	+ + +	+	+	+	+ + +	–
Swine	+	+	+	+	+	–
Horse	+ +	+	+ +	+	+	–
Sheep	+	+	+	+	+	–
Human	+ +	+	+	+	+ +	–
Fowl	–	–	–	–	–	–
Fibrinogen						

* Explanation in the text.

The results of absorption experiments are shown in Table III.

The data of the Table clearly demonstrate that swine fibrinogen antiserum is absorbed by any of the other mammalian fibrinogens. The exhausted antiserum causes a precipitation of the homologous fibrinogen, but in a lower titre only. No absorption of the swine fibrinogen antiserum is attained by fowl fibrinogen, as examined with the homologous antiserum. Using the other antisera, identical results were obtained.

[1] P. R. MORRISON, J. Amer. Chem. Soc. *69*, 2723 (1947).
[2] J. E. GAHRINGER, J. Immunol. *12*, 477 (1926).

Table III

Absorption test with heterologous fibrinogens

Antibodies	Antigens				
	Beef	Swine	Horse	Sheep	Human
	Fibrinogen				
Swine fibrinogen antiserum + phys. NaCl*	1:125 +	1:250 +	1:125 +	1:125 +	1:125 +
Swine fibrinogen antiserum + Beef fibrinogen	–	1:32 +	–	–	–
Swine fibrinogen antiserum + Horse fibrinogen	–	1:64 +	–	–	–
Swine fibrinogen antiserum + Sheep fibrinogen	–	1:64 +	–	–	–
Swine fibrinogen antiserum + Human fibrinogen	–	1:64 +	–	–	–
Swine fibrinogen antiserum + Fowl fibrinogen	1:125 +	1:250 +	1:125 +	1:125 +	1:64 +
Swine fibrinogen antiserum + Swine serum	1:125 +	1:250 +	1:125 +	1:125 +	1:64 +

* The antiserum was mixed in each case with an equal part of phys. NaCl, or of fibrinogen solution, or of serum.

Our experiments demonstrate that a *very near relationship exists between the examined mammalian fibrinogens. Though all of them have some slight species specificity, there is a marked immunological boundary between mammalian and fowl* fibrinogen. Analytical studies concerning the polysaccharide content of various fibrinogens may indicate also the existence of such a difference[1] demonstrating *the presence of identical saccharides in mammalian fibrinogens and slight differences only in the ratio of hexose/glucosamin. The glucosamin level is considerably higher in fowl fibrinogen and fibrin.* Analytical data of this kind are of importance with regard to our problem too, since it is wellknown that in carbohydrate-protein complexes the carbohydrates play an important role in determining antigen properties.

Further investigations are in progress. Full details will be published elsewhere.

D. BAGDY and T. SZILÁGYI

Institute of Pharmaceutical Industrial Research, Budapest, and Institute of Pathophysiology, Medical University, Debrecen, December 1, 1952.

Zusammenfassung

Kaninchen wurden nach der Methode von LAKI und BAGDY mit lyophilisierten Fibrinogenen vom Rind, Schwein, Pferd, Schaf, Geflügel und Mensch intravenös immunisiert. Die Säugetier-Fibrinogen-Immunsera *präzipitierten* nicht bloss das homologe Fibrinogen, sondern auch die übrigen Säugerfibrinogene; sie reagieren jedoch mit dem Geflügelfibrinogen nicht. Das Geflügelfibrinogen-Antiserum reagiert mit keinem Säugetierfibrinogen. Es wurde festgestellt, dass an mit Säugerfibrinogen sensibilisierten Tauben die Injektion eines anderen Säugerfibrinogens ebenfalls anaphylaktische Erscheinungen auszulösen imstande ist. Säugerfibrinogen-Antisera lassen sich durch das Fibrinogen eines anderen Säugetieres absättigen; das abgesättigte Immunserum bewirkt nur mehr eine Präzipitation des homologen Fibrinogens bis zu einem niedrigeren Titer. Durch Geflügelfibrinogen und homologes Serum lässt sich hingegen das Fibrinogen-Antiserum nicht absättigen. Die chemischen Analysen ergaben zwischen dem Kohlenhydratgehalt des Säuger- und Geflügelfibrinogens messbare Unterschiede.

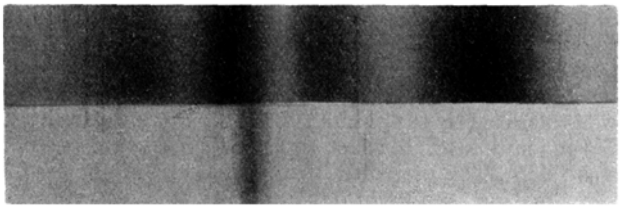
[1] I. SZÁRA and D. BAGDY, Exper. (in press).

Über das Verhalten der Serumcholinesterase des Pferdes bei der Papierelektrophorese

Einleitung. AUGUSTINSSON[1] schreibt der Serumcholinesterase Albumincharakter zu. Klinische Beobachtungen von FABER[2] weisen auch in diese Richtung. Dazu im Widerspruch stehen die Resultate von GLICK, GLAUBACH und MOORE[3], die auf elektrophoretischen Untersuchungen basieren. Danach zeigt die Cholinesterase Beziehungen zur α- und vor allem zur β-Globulin-Unterfraktion.

Die vorliegende Mitteilung soll einen weiteren Beitrag zur Abklärung dieser Frage liefern.

Methoden. Um festzustellen, mit welcher Proteinfraktion sich die Serumcholinesterase im elektrischen Feld bewegt, wurde das Serum zuerst durch Elektrophorese auf Filtrierpapier in seine Fraktionen aufgetrennt, wobei wir uns im Prinzip an die Anordnung von GRASSMANN und HANNIG[4] hielten. Darauf lokalisierten wir die Cholinesterase-Aktivität im Filtrierpapierstreifen durch Anwendung der histochemischen Methode von KOELLE und FRIEDENWALD[5].



Die Abbildung zeigt einen der Elektrophorese unterzogenen Filtrierpapierstreifen. Dabei wurde die obere

[1] K.-B. AUGUSTINSSON, Ark. Kemi Miner. Geol. [A] *18*, Nr. 24 (1944); Acta Physiol. Scand. *15*, Suppl. 52, 25 (1948).

[2] M. FABER, Acta Med. Scand. *114*, 72, 475 (1943).

[3] D. GLICK, S. GLAUBACH und D. H. MOORE, J. Biol. Chem. *144*, 525 (1942).

[4] W. GRASSMANN und K. HANNIG, Naturwiss. *37*, 496 (1950); Hoppe-Seylers Z. phys. Chem. *290*, 1 (1952).

[5] B. KOELLE und J. S. FRIEDENWALD, Proc. Soc. Exp. Biol. Med *70*, 617 (1949).