SUMMARY

1. By taking advantage of the low metabolic rate of the alligator, it was possible to study the effect of insulin on the rate at which each of a large group of amino acids was utilized *in vivo*.

2. 'Amino acid tolerances' of both isomers of amino acids were determined in the alligator and in the rat by the injection of 10 m-moles/kg. followed by analyses of plasma amino acids at frequent intervals. The rat metabolized amino acids from 50 to 100 times as fast as the alligator. In general, the non-essential amino acids were utilized much more rapidly than the essential ones by both species.

3. Each amino acid had its characteristic tolerance curve.

4. In the alligator, insulin increased the rate of metabolism of most of the exogenous amino acids of both isomers.

5. Insulin appears to enhance the metabolism of compounds which differ greatly in chemical structure.

We wish to thank Mr Charles R. Martinez and Mrs Jessie Hano for technical assistance and Mr N. J. Nicosia for preparation of the figures. This research was supported in part by a grant from the National Institutes of Health (No. H-2062).

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Biochem. J. (1961) **79**, 605

The Effect of Testicular Hyaluronidase on the Hyaluronic Acid Complexes of Human Synovial Fluid

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(Received 11 November 1960)

It has been shown that the electrophoretic mobility of the human synovial-fluid proteins is affected by the viscosity of the solution (Pigman, Patton & Platt, 1957). This effect was interpreted as a formation of a complex of the viscous component of synovial fluid, hyaluronic acid, with the proteins. Evidence for this formation has been given by a number of investigators (Ropes, Robertson, Rossmeisl, Peabody & Bauer, 1947; Ogston & Stanier, 1950, 1952; Curtain, 1955; Blumberg & Ogston, 1957; Pigman *et al.* 1957; Hamerman & Sandson, 1959; Pigman, Gramling, Platt & Holley, 1959). A component moving with

a mobility between those for hyaluronic acid and albumin was found in many normal and post-mortem human synovial fluids (Platt, Pigman, Holley & Patton, 1956; Pigman *et al.* 1957). This component has been named the π -peak (Platt *et al.* 1956) and was believed to be a stable complex between hyaluronic acid and protein (Platt, 1957). The present investigation was undertaken to ascertain whether the π -peak is a non-dissociable complex between hyaluronic acid and protein, and whether the increase in the electrophoretic mobility of the synovial proteins is solely the result of an increase in viscosity.

MATERIALS AND METHODS

Human synovial fluids. The human synovial fluids were aspirated *post mortem* from the knee joint at hospital autopsy within a few hours after the death of the patient. All fluids were obtained from individuals with no history of joint symptoms or arthritic disease. When the amount of fluid was deemed insufficient for electrophoretic analysis, two or more fluids were pooled. The samples were placed in sterile containers and kept under refrigeration until they were analysed.

Hyaluronidase treatment. Two types of enzymic treatment were used: for mild treatment with hyaluronidase, the synovial fluids were treated with 2 vol. of buffer (pH 8.6, see below) and incubated at 30° for 2 hr. after the addition of 1 T.R.U. (turbidity reducing unit) of testicular hyaluronidase/ml. of synovial fluid. During this period of time, the solution was shaken vigorously for 1 min. at 15 min. intervals. The sample was then dialysed against 500 ml. of buffer for 48 hr. at 2-4°. For more drastic treatment with hyaluronidase, 3 T.R.U. of hyaluronidase/ml. of synovial fluid was added to the undiluted synovial fluid. The solution was incubated at 37° for 2 hr. and shaken vigorously at 15 min. intervals. At the end of the incubation period, the fluid was diluted with 2 vol. of buffer and dialysed as described above. The buffer used was sodium diethyl barbiturate (pH 8.6, *I* 0.1).

The testicular hyaluronidase (1250 T.R.U./mg.) was obtained from Wyeth Laboratories, Philadelphia, Pa., U.S.A.

Electrophoretic analysis. The fluids after dilution and dialysis were analysed by using a Perkin-Elmer Tiselius-type electrophoresis apparatus with the 2 ml. cell at 0.5°, with a constant current of 6 ma. The time of the runs ranged from 6000 to 8200 sec. The electrophoretic mobilities were calculated from the ascending patterns for reasons previously described (Platt *et al.* 1956; Pigman *et al.* 1957).

Viscometry. Viscosity measurements were made at 30° with two types of viscometers, a pipette-type viscometer (Pigman *et al.* 1957) and Cannon-Manning capillary semi-microviscometers (Cannon Instrument Co., State College, Box 812, Pa., U.S.A.).

RESULTS

The results of the electrophoretic analyses of human synovial fluid before treatment with testicular hyaluronidase are shown in Table 1. Of the ten fluids analysed, all contained hyaluronic acid, albumin and α_1 - and β -globulin. Six of the samples had a detectable α_2 -globulin component, and four a γ -globulin component. A π -peak was present in the patterns for four fluids. Hyaluronic acid always moved as a sharp peak. The viscosity range of the fluids was 6.3-30.8 cp. All protein components with the exception of γ -globulin exhibited a mobility which increased with the viscosity of the solution.

After treatment with hyaluronidase, the viscosities of the fluids dropped markedly, as shown in Table 2. The viscosity ranged from 0.9 to 4.7 cp. All the samples contained hyaluronic acid, albumin and α_1 - and β -globulin. In eight of the fluids the α_2 -globulin peak could be detected, and in seven a γ -globulin component was present; in some of these fluids these components were demonstrated only after the enzyme treatment. The π -peak which was found in four of the fluids before hyaluronidase treatment could not be detected in any subsequent to the treatment. The profile of the hyaluronic acid peak appeared to be only slightly affected by enzymic treatment, which produced a broadening of the base of the peak.

The mobility of the hyaluronic acid was essentially unchanged by treatment with hyaluronidase. The mobilities of the synovial proteins decreased markedly after the action of hyaluronidase. Although the greatest viscosity of the treated fluids was five times as great as that of the least viscous fluids, there was little variation in the mobilities of the individual components in the enzyme-treated fluids. The mobilities of the protein

Table 1. *Electrophoretic mobilities of human synovial-fluid components*

The values in columns 2-8 are given as $-10^5 \times$ electrophoretic mobility ($\text{cm}^2 \text{v}^{-1} \text{sec}^{-1}$). — Indicates not found.

Fluid	Hyaluronic acid	π	Albumin	Globulins				Viscosity (centipoises)
				α_1	α_2	β	γ	
A	9.3	—	7.3	6.5	5.2	3.7	1.5	6.3
B	9.1	—	7.4	6.6	—	4.0	1.8	9.0
C	9.5	—	7.6	6.2	—	4.7	—	14.5
D	9.5	8.0	7.7	7.1	—	4.6	—	14.5
E	9.2	8.2	7.8	7.2	5.5	4.7	—	16.7
F	9.2	8.4	7.9	7.2	5.7	5.0	2.1	20.3
G	9.0	—	8.1	7.1	5.8	5.1	—	23.7
H	9.1	8.6	8.2	7.2	—	5.3	—	24.6
I	9.0	—	8.4	7.0	6.0	5.6	—	27.2
J	9.4	—	8.8	7.3	6.4	5.9	1.8	30.8
Average	9.3	8.3	7.9	7.0	5.8	4.9	1.8	—
Range	9.0-9.5	8.0-8.6	7.3-8.8	6.2-7.3	5.2-6.4	3.7-5.9	1.5-2.1	—
Serum*	—	—	6.4	5.5	4.5	3.5	1.4	0.9

* Pigman, Patton & Platt (1957).

Table 2. *Electrophoretic mobilities of human synovial-fluid components after treatment with testicular hyaluronidase*

The values in columns 2-7 are given as $-10^5 \times$ electrophoretic mobility ($\text{cm.}^2 \text{v}^{-1} \text{sec.}^{-1}$). The first letter denoting the fluid analysed corresponds to the letter in Table 1. (The final letter H refers to treatment with hyaluronidase.)

Fluid	Hyaluronic acid	Albumin	Globulins				Viscosity (centipoises)
			α_1	α_2	β	γ	
AH	8.9	6.4	5.4	4.7	3.4	1.8	0.9
BH	9.1	6.4	5.5	4.5	3.4	1.7	1.3
CH	9.1	6.6	5.6	—	3.7	—	1.6
DH	9.0	6.7	5.7	5.0	3.8	1.4	4.4
EH	9.3	6.6	5.5	4.6	3.7	1.6	1.2
FH	9.1	6.5	5.6	4.7	3.7	1.7	4.6
GH	9.1	6.6	5.6	4.7	3.6	1.4	4.2
HH	9.3	6.5	5.4	4.6	3.5	—	1.8
IH	9.1	6.4	5.6	—	3.8	—	4.7
JH	9.0	6.6	5.6	4.7	3.7	1.7	4.7
Average	9.1	6.5	5.6	4.7	3.6	1.6	—
Range	8.9-9.3	6.4-6.7	5.4-5.7	4.5-5.0	3.4-3.8	1.4-1.8	—
Serum*	—	6.4	5.5	4.5	3.5	1.4	0.9

* Pigman *et al.* (1957).

components for the treated fluids were similar to that obtained for blood serum, which has a viscosity of 0.9-1.0 cp under similar conditions. γ -Globulin appeared to be the least affected by treatment with hyaluronidase.

The disturbances often noted in the descending limb when the untreated samples were analysed were not present in the hyaluronidase-treated fluids. The electrophoretic patterns appeared to be 'sharper' in the hyaluronidase-treated fluids except for the hyaluronic acid component. A slight decrease seemed evident in the albumin/globulin ratios after treatment with hyaluronidase, but this decrease did not appear to be significant.

DISCUSSION

It was postulated that the increase in mobilities of the synovial-fluid proteins that occurs with an increase in the viscosity of that fluid may arise from complex-formation between hyaluronic acid and the synovial proteins (Pigman *et al.* 1957). Curtin (1955) showed that the hyaluronic acid complex, isolated by ultrafiltration from synovial fluid, on electrophoresis contained, in addition to hyaluronic acid, proteins which behaved similarly to the major serum components.

The loss of the π -component on treatment with hyaluronidase suggests that it consists of hyaluronic acid loosely associated with proteins. In other work (Pigman, Gramling & Holley, 1960), π -components have been formed in mixtures of highly polymerized hyaluronic acid and serum albumin under the conditions of the present work. Katchalsky (1954) has indicated that the electrostatic fields of macromolecules are sufficiently

powerful to permit the cross-linking of macromolecules by electrovalent bonds, despite the dissociative action of water resulting from its high dielectric constant. This concept suggests that the ionized carboxyl groups of hyaluronic acid can react directly with the cationic amino groups of the proteins. Even in the virtual absence of charged groups in one polymer, the interaction between a polyelectrolyte and a 'neutral' polymer resulted in the formation of a new component in the mixture (Platt, 1960). The complex formed between sodium carboxymethylcellulose and the 'neutral' polymers, methylcellulose and polyacrylamide, was stable in an electrical field.

The degree of polymerization of hyaluronic acid seems to be important in complex-formation. The apparent effect of viscosity on the electrophoretic mobilities of the proteins suggests a direct correlation between mobility and viscosity. However, the viscosity of some of the fluids treated with hyaluronidase was similar to that of untreated fluids in the earlier study (Pigman *et al.* 1957), but, in the hyaluronidase-treated fluids, no effect of viscosity on mobility could be detected. This result indicates that degradation of the hyaluronic acid molecule resulted in a breakdown of the hyaluronic acid complex and that partially depolymerized hyaluronic acid will not form the complex. That high-molecular-weight hyaluronic acid is required for the formation of the π -complex was also demonstrated by the earlier failure to produce a π -peak by the addition of low-molecular-weight hyaluronic acid to synovial fluid and blood serum (Platt, 1957).

In contrast with the electrophoretic work, testicular hyaluronidase has been shown to exert little

or no effect on the sedimentation behaviour of synovial fluid (Platt, Pigman & Holley, 1959) and the hyaluronic acid complex (Fessler, 1960).

SUMMARY

1. Human synovial fluid was analysed electrophoretically before and after treatment with testicular hyaluronidase.

2. After hyaluronidase treatment, the electrophoretic mobilities of the synovial proteins decreased markedly and were similar to the values obtained for blood serum proteins.

3. The π -component, a complex of hyaluronic acid and albumin, could not be detected after hyaluronidase treatment.

This work has been supported by grants from the National Institute of Arthritis and Metabolic Diseases (A-1225) and the John R. Irby Fund for the study of Rheumatic Disease. One of us (D.P.) wishes to thank the Arthritis and Rheumatism Foundation for a fellowship during most of the period of this work.

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Biochem. J. (1961) **79**, 608

The Control of some Oxidative Pathways in Guinea-Pig Mammary-Gland Mitochondria

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(Received 26 October 1960)

The control of metabolic pathways can occur on three different time scales. First, the steady-state levels of metabolite concentrations can be changed within seconds by the activation or inhibition of enzymes due to the introduction of metabolites, hormones or other modifiers. These rapid changes can involve the release of preformed enzymes but are not likely to involve synthesis of enzymes. The second time scale covers a long range from minutes to days, which are the periods required for adaptive enzyme formation. In this paper we are concerned with a third and very much longer time scale required for tissue to adapt itself to a special purpose during the evolution of the organism. For the biosynthesis of proteins and fatty acids, the mammary gland is the specialized organ *par excellence*. Turner (1951) has already shown that the tricarboxylic acid cycle functions in mammary tissue. The present work was undertaken as part of a study of the biochemistry of the mammary gland. We are also interested in examining mito-

chondria, from specialized tissue, for quantitative differences in enzyme activities of steps of the citric acid cycle, and of steps involved in the supply and utilization respectively of substrates and products of the cycle. A study of a wide range of oxidative and other metabolic reactions can be shown to reflect the predominantly anabolic activity of the mammary gland.

Many different criteria have been formulated to define intact mitochondria. We have relied upon the evaluation of the P/O ratio (phosphorylation of adenosine diphosphate to adenosine triphosphate/oxygen uptake) in the presence of different substrates and of respiratory control (respiration in the presence and absence of the phosphate acceptor, adenosine diphosphate) (Lardy, 1956) as a measure of the biochemical integrity of our mitochondrial preparations. By far the largest number of investigations on mitochondria has been carried out with preparations from liver. From this source good preparations can be obtained with relative ease by