Protein content and glycolitic activities of human amniotic fluid

Samples	Protein[1]	Aldolase[2]	PEI[3]	LDI[4]	PRI[5]
1	208	0·278	0·340	0·250	—
2	296	0·094	0·108	0·054	—
3	206	0·237	0·340	0·155	—
4	140	0·121	—	—	—
5	262	0·244	0·150	0·056	—
6	193	0·057	0·052	0·088	0·045
7	164	0·025	0·045	0·079	0·095
8	148	0·048	0·018	0·034	—
9	255	0·040	0·097	0·029	0·149
10	241	0·149	0·059	0·021	—
11	302	0·087	0·108	0·043	0·023
12	182	0·312	0·185	—	—
13	145	0·067	0·140	0·137	0·034
14	200	0·055	0·106	0·067	0·318
15	160	0·056	0·090	—	0·220
16	206	—	0·106	—	0·089
17	205	0·024	—	—	0·263
18	162	0·019	—	—	0·405
19	175	0·103	—	—	0·240
20	217	0·032	—	—	0·350
21	207	0·034	—	—	0·130
Mean values	203 ± 46	0·104 ± 0·091	0·149 ± 0·101	0·181 ± 0·126	0·085 ± 0·066

[1] Protein = mg%ml.
[2] Aldolase = μM hexosediphosphate/h/mg of protein.
[3] PEI = phosphohexoso-isomerase = mg fructose/h/mg of protein.
[4] LDI = lactic-dehydrogenase = μM/min/mg of protein.
[5] PRY = phosphoribose-isomerase = μM ribulose/h/mg of protein.

was determined with the sulfuric acid-cistein-carbazole reagent.

Lactic dehydrogenase.—This activity was determined spectrophotometrically[12] as follows: in a cuvette of the Beckman spectrophotometer were pipetted: 0·4 μM DPNH in 0·1 ml, 3 μM piruvate in 0·1 ml, 0·3 ml $NaHCO_3$ 0·02 M, 2·5 ml phosphate buffer 0·1 M pH 7·8.

The reaction was started by addition of 0·1 ml amniotic fluid and the decrease of O.D. at 340 mμ was followed for 10 min at room temperature taking readings every minute.

In the Table the data obtained in these experiments are reported with their mean and standard deviation. It is evident that all enzymatic activities tested are present in considerable amounts in the amniotic fluid.

The enzymatic activity referred to the protein content is much higher in amniotic fluid than in blood serum of either mother or newborn[13].

The values obtained from the different samples are considerably scattered. It has not been possible to relate such differences to the way in which the fluid was obtained nor the time the liquid have been stored.

The protein values obtained are in good agreement with those previously reported by other authors[14].

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Riassunto

È stata messa in evidenza nel liquido amniotico di donne a termine di gravidanza la presenza di alcuni enzimi glicolitici. Sono riportati i dati quantitativi ottenuti per l'attività della fosforiboso-isomerasi, aldolasi, fosfoesoso-isomerasi e lattico-deidrogenasi. È stato determinato inoltre il contenuto proteico dei campioni di liquido amniotico presi in esame.

Post Nephrectomy Increase in Serum Ribonuclease Activity after Total Hepatectomy or Nitrogen Mustard Derivatives Administration*

It has been previously shown that serum ribonuclease (Rase) activity markedly increases after bilateral nephrectomy in the rat[1], this phenomenon being observed in animals undergoing a number of experimental procedures, including 'functional evisceration'[2]. Although our results suggested that the liver is not the source of the enzyme increase, the disadvantages of leaving that organ *in situ*[3] led us to study the effects of total hepatectomy and evisceration. These were performed by the two stage technique of INGLE[4] on Wistar rats of both sexes (average body weight 250 g). The second stage was carried out under ether anesthesia, and was immediately followed by bilateral nephrectomy, non-nephrectomised

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animals serving as controls. The rats were kept at 27°C after operation, there being an average rectal temperature drop of 4°C. Blood was obtained from the jugular veins at nephrectomy and 4 h later.

The presence of Rase in leucocytes[5], and the increase in urine Rase activity in myeloid leukemia[6], prompted us to investigate whether in granulopenic animals the same effect of bilateral nephrectomy would be obtained. For that purpose, rats of both sexes, 200–250 g body weight, received either 2 mg/kg of methyl-bis-β-chloroethylamine hydrochloride[7] ('Dichloren' Ciba) intravenously, or two 20 mg/kg doses of 1–4 dimethanesulfonoxybutane[8] ('Myleran' Burroughs Wellcome) intraperitoneally 5 days apart. The 'Dichloren' treated rats were nephrectomised on the 5th day, and the rats treated with 'Myleran' on the 12th day after the initial administration of the drugs. The animals were bled at the start of the experiments, at the time of nephrectomy and 4 h later. Comparison of samples of the first two stages showed that both drugs had no effect on serum Rase activity. Total and differential white blood cell counts were obtained from these animals as well as from the controls.

Results are to be found in the Tables I and II.

It can be seen that in the eviscerated-totally hepatectomized animals, and in the animals treated with the nitrogen mustard derivatives, the increase in serum Rase activity after nephrectomy is not significantly different from that of control animals. The pronounced lymphoid aplasia observed in the 'Dichloren' treated rats gives support to our assertion[1] that lymphoid tissue is presumably not involved in the post-nephrectomy increase in serum Rase activity.

The above data, together with those reported previously, tend to rule out, therefore, either the liver, pancreas, digestive tract, adrenals or hemopoietic tissues, as the sole sources of the enzyme increase. Nevertheless, we cannot exclude the possibility that these, together with other tissues and organs, release the enzyme into the blood, so that their individual contribution would be undetectable under the present conditions, for each of them would then represent but a small fraction of the total mass of the body.

Table I

Serum Rase activity in hepatectomised-eviscerated rats with and without bilateral nephrectomy

Treatment	Number of animals	Serum ribonuclease activity*
Hepatectomised + evisceration . . .	5	97·9 ± 18·6
Hepatectomised + eviscerated + bilat. nephrectomy	8	161·0 ± 10·7

* Mean in % of pre-nephrectomy activity ± S.E.

Table II

Total leucocyte counts, total neutrophil counts and serum Rase activity in rats nephrectomised after N mustard derivatives treatment

Treatment	No. of animals	Total leucocyte counts[1]	Total granulocyte counts[1]	Serum Rase activity[2]
Controls . .	8	125·0 ± 15·3[3]	127·3 ± 16·2	229·9 ± 17·7
'Dichloren' .	5	6·4 ± 2·5	7·9 ± 3·7	274·4 ± 16·8
'Myleran' . .	4	27·0 ± 3·5	13·9 ± 4·9	204·2 ± 23·8

[1] Counts taken immediately before nephrectomy in % of initial counts before drug administration.
[2] In % of pre-nephrectomy activity.
[3] Mean ± S.E.

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Résumé

L'augmentation de l'activité de la ribonucléase du sérum, qui fait suite à la néphrectomie bilaterale chez le rat n'est pas modifiée par une évisceration préalable avec hépatectomie totale ou administration de moutardes azotées. Ces résultats indiquent que ni le foie ni les leucocytes et organes hémopoiétiques ne peuvent être considerés comme sources exclusives de cette augmentation.

Antibodies Against Connective Tissue in Collagen Diseases

Autoimmunizing processes are reported as possible factors in the pathogenesis of acute rheumatism and recently in other diseases included among collagen diseases[1]. It is natural that attention was also directed to the ground substance. A component of the ground substance—the hyaluronic acid—in the tests of some authors proved to be nonantigenic[2]. As noted by Seifter[3], Tinacci and Benassi recently ascertained an antigenic response in rats and rabbits after a single dose of purified hyaluronate prepared from human umbilical cords. An attempt was made to ascertain whether autoantibodies, reacting with isolated components of the ground substance, develop in collagen diseases. From the series of constituents of ground substance, attention was first paid to hyaluronic acid. Since the hyaluronic acid-hyaluronidase system is striking disorganized in febris rheumatica, the question was first investigated in this disease.

Material and Methods.—The sera of the rheumatic patients and controls were stored at −25°C and elaborated within 72 h of collecting the blood. Hyaluronic acid (HA) was prepared in the form of potassium hyalur-

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