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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onate by the method of McCLEAN[4] in the modification of HARRIS *et al.*[5].

Antibodies against hyaluronic acid were demonstrated either by precipitation in capillary tubes or by haemagglutination with unprepared erythrocytes or by collodion agglutination. The results obtained by collodion agglutination only are given since this particular method proved to be the most suitable. The collodion agglutination was performed by the method of CAVELTI[6] in the modification of WAGNER[7]. For the collodion agglutination the hyaluronic acid was dissolved in physiological saline in the ratio of 5 mg to 1 ml of the solvent under sterile conditions at 37°C. All the tests were performed with one batch of HA. The reaction was read off by tapping the bottom of the test-tube while observing it with side illumination. Positive reactions are characterized by forming floccules which do not break up on shaking, and negative reactions by diffuse turbidity. The test was appraised only if all the controls (antigen with collodion, serum with collodion, serum of a healthy person with antigen and collodion) were completely negative.

Results.—The sera investigated were divided into two groups. The first group consisted of 127 healthy subjects for the most part blood-donors. None of the control sera gave a positive result in collodion agglutination with HA. In the second group 273 sera from 115 patients hospitalized with a still active form of febris rheumatica were examined. The latter group includes cases with different stages of the disease and with varying activity of the pathological process. These sera were positive in the collodion agglutination with HA in 59% of the examined sera, i.e. the titre was 1:4 and higher. Most frequently achieved titres were 1:8 up to 32.

In the latter group we compared the results achieved with HA as antigen with the results of the other nonpurified tissue antigens (connective tissue, skin, myocardium, brain, bone-marrow, liver, spleen). On account of the negative controls in the former group, it was possible to combine the results in two groups to get greater numbers: one with negative results, the other with positive results. The dependence was appraised by the χ^2-test. The results are shown in the Table.

The hypothesis of independence between HA- and organ-antibodies was disproved for myocardium, connective tissue and skin only. All values of χ^2 are highly significant. By selecting the material into groups according to the titre 0, 4–16, 32 and more, the same significance was ascertained as in the collected material. For the other antigens employed (bone-marrow, liver, spleen, brain) the values of χ^2 are not significant on 5% level and thus the hypothesis of independence cannot be rejected.

Discussion.—By means of collodion agglutination, autoantibodies against one of the components of the ground substance – the hyaluronic acid – were successfully demonstrated in the serum of patients with febris rheumatica. A comparison of the serum reaction with HA and with other nonpurified tissue antigens showed a dependence directly proportional to the content of HA in the tissue concerned: the highest dependence found, between the skin antigen and HA, seems to be conditioned by the high concentration of HA in the skin unlike the connective tissue and myocardium where its content is essentially lower and unlike further organs where it is found in insignificant concentrations[8]. The negative results with HA with the simultaneous positive finding with an other nonpurified connective antigen show, however, that even other components than HA can be affected by the immunotoxic mechanism in collagen diseases.

Occurrence of Autoantibodies against Hyaluronic Acid and Myocardium, Connective Tissue and Skin in Rheumatic Fever.

Hyaluronic Acid	Myocardium			Connective Tissue			Skin		
	–	+	Total	–	+	Total	–	+	Total
–	91 (72·3)	16 (34·7)	107	92 (64·6)	12 (39·3)	104	92 (54·7)	20 (57·3)	112
+	90 (108·7)	71 (52·3)	161	69 (96·3)	86 (58·6)	155	30 (67·3)	108 (70·7)	138
Total	181	87	268	161	98	259	122	128	250

At present, however, no definite statement can be made as to what kind of hyaluronic acid acts as the primary antigen in autoimmunization in rheumatism – the hyaluronic acid originating in the tissue proper or that originating in the streptococcus-capsules. It cannot be also stated whether HA-antibodies cause changes that are manifested e.g. by precipitation by being bound to tissue HA. The role of autoantibodies in the development of fibrinoid necrosis presents at present only a working hypothesis awaiting its confirmation by model tests.

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Zusammenfassung

In Seren *Febris rheumatica*-Kranker wurden Autoantikörper gegen isolierte Hyaluronsäure durch Kollodion-Agglutination gefunden.

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