

SCHEIDEGGER² on microscopic slides covered with 2 ml of gel in barbiturate buffer (pH 8.6, 0.05 μ). Antiserum against dog plasma was prepared by injection into rabbits. The samples were also fractionated by paper electrophoresis.

Nine experiments were performed. Eight samples of hepatic, intestinal and cervical, four samples of renal and two of thoracic duct lymph were analysed. No particular differences were disclosed by paper electrophoresis in the composition of lymph samples of different origin and of blood plasma. The same protein fractions were present in lymph and blood plasma and only minor quantitative differences could be detected. By immunoelectrophoretic analysis of the liver lymph, however, several precipitation lines, corresponding to α - and β -globulin fractions, could be observed, which were present neither in the lymph samples of other origin nor in the plasma of the same animal.

As is well known, the liver plays an important part in the production of plasma proteins. It can be assumed, therefore, that proteins formed by the liver are at least partly transported to the general circulation by the lymphatics. Protein components formed in minimal quantities are strongly diluted in the blood plasma and cannot

be detected there even by the highly sensitive immunoelectrophoretic method.

Our investigations present evidence to the hepatic production of such protein components, but do not permit their exact identification.

Zusammenfassung. Vergleichende immunoelektrophoretische Untersuchungen des Blutplasmas, der Nieren-, Leber-, Darm- und Tr.-cervicalis-Lymph zeigten mit Ausnahme der Leberlymph in den Lymphproben die gleichen Proteinfractionen wie im Blutplasma. Im Immunoelektrophorogramm der Leberlymph waren an den Stellen der α - und β -Globulinfractionen mehrere Präzipitinstreifen sichtbar, die im Plasma bzw. in den Lymphproben anderen Ursprungs nicht vorhanden waren.

GY. SZABÓ, J. GERGELY, and ZS. MAGYAR

National Institute of Traumatology, First and Third Departments of Medicine, University Medical School, Budapest (Hungary), October 4, 1962.

² J. J. SCHEIDEGGER, *Int. Arch. All.* 7, 103 (1955).

Changes in Vascular Permeability and Dermal Mucopolysaccharides in the Female Rat after Parathyroidectomy

In the parathyroidectomized female rat the ovary can compensate for the parathyroid function in the homeostasis of plasma calcium¹⁻⁴, probably by stimulating the calcium absorption. Nevertheless, the survival period of parathyroidectomized female rats is generally shorter than that of the control animals, and death ensues from banal infections to which the operated rats become particularly susceptible. Infection seems to be facilitated either by decrease of immunological reactions or by easier penetration and spreading of the infectious agents. It was also observed that the inflammatory reaction—and subsequent necrosis of the skin—produced experimentally by subcutaneous injections of croton oil in the parathyroidectomized female rats is wider spread in these animals than in the controls. This has been explained by the hypothesis that in the operated rats a higher tissue permeability was induced by changes in the calcium metabolism⁵. Previous works have shown an influence of the parathyroids on connective tissue: bone decalcification provoked by hyperparathyroidism is associated with modifications of the organic bone matrix and this is believed to be the primary action of the parathyroid hormone⁶⁻⁸. A modification of the mucopolysaccharide components of serum has also been described⁹.

In the present paper, experiments on the effects of the parathyroidectomy on the vascular permeability and on the connective tissue are described.

Female Sprague-Dawley rats were parathyroidectomized when weighing 150–200 g. In the first experiment vascular permeability changes were investigated by the method of WILHELM et al.¹⁰. The experiment was carried out on 9 parathyroidectomized rats, 15, 30 and 60 days after the operation, and on 10 controls (some of them subjected to sham-operation). The abdomen of the rats was accurately shaved 48 h before the test; 0.2 ml of 5%

solution of Pontamine Sky-Blue 6 BX (Gurr's) were injected intravenously into the tail or the jugular veins. Half an hour later, 0.1 ml of rat serum was injected intradermally into the shaved area, in order to provoke an inflammatory reaction, induced by the plasma factor (P.F./native) of WILHELM et al.¹⁰. This mild inflammatory agent was chosen in order to follow the evolution of the inflammatory reaction more easily. The changes in permeability of the capillaries were evaluated according to the time taken for the skin to stain and the extension and intensity of the stain around the site of injection of the rat serum. The spreading of the colour around the injection site begins earlier in the parathyroidectomized than in the control rats: 1 h after the injection the spreading of the colour and its intensity is definitely greater in the operated rats (Figure).

A difference was already noticed in the animals operated 15 days before; it was more marked in the groups operated 30 and 60 days before.

The animals were subsequently bled in order to determine the calcium content in serum, after which they were dissected; a final evaluation of the skin reaction was made

¹ P. ROWINSKI and G. MANUNTA, *Boll. Soc. Ital. Biol. sper.* 27, 37 (1951).

² P. ROWINSKI and G. MANUNTA, *Rend. Acc. Naz. Lincei* 10, 495 (1951).

³ G. MANUNTA, *Rend. Acc. Naz. Lincei* 12, 104 (1952).

⁴ A. MILLO and A. BEGLIOMINI, *Riv. di Biol.* 50, 405 (1958).

⁵ G. MANUNTA, *Riv. di Biol.* 50, 279 (1958).

⁶ I. GERSH and H. R. CATCHPOLE, *Amer. J. Anat.* 85, 457 (1949).

⁷ MINNIE HELLER-STEINBERG, *Amer. J. Anat.* 89, 347 (1951).

⁸ M. B. ENGEL and H. R. CATCHPOLE, *Proc. Soc. exp. Biol. Med.* 84, 336 (1953).

⁹ M. R. SHETLAR, R. H. BRADFORD, W. JOEL, and R. P. HOWARD, *The Parathyroids*. Proceedings of a Symposium on Advances in Parathyroid Research (Charles C. Thomas Publ., Springfield 1961), p. 144.

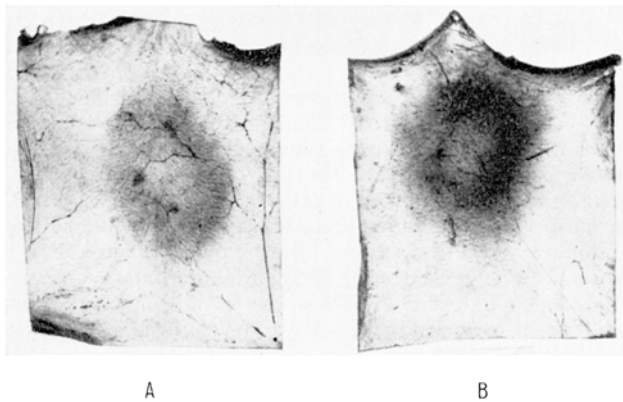
¹⁰ D. L. WILHELM, P. Y. MILL, ELIZABETH M. SPARROW, MARGARET E. MAC KAY, and A. A. MILES, *Brit. J. exp. Path.* 39, 228 (1958).

on the inner side of the skin. The muscles, liver and intestine of the operated animals were also more distinctly coloured than those of the controls.

Calcemia in the parathyroidectomized animals was 10.0 ± 1.3 mg%, in the controls 10.8 ± 1.2 %; these normal values in the operated animals were somewhat surprising, though, as has been noted^{1,2}, tetany does not develop in female rats on a normal diet. As the calcium content of our diet was very high (2.9%) this fact might explain these results. It is extremely unlikely that accessory parathyroids would be present in all the operated cases.

Pontamine-Blue concentration in the serum was also determined in order to exclude the possible colour differences in the operated and control rats originating from different stain concentrations in the blood: no statistically significant differences were observed.

In the second experiment the skin of the left side of the abdomen of 29 rats (15 controls and 14 parathyroidectomized 15, 30, and 60 days before) was fixed in Bouin's fluid. Paraffin sections were stained with Toluidine-Blue (Grübler). A 1/5000 solution of the dye was prepared with Mc Ilvaine's buffer (pH 5.4) diluted 1/10.



Spreading of the colour, 1 h after the intradermal injection of rat serum. Inner side of the skin of the abdomen. – (A) control rat; (B) parathyroidectomized rat.

The development of metachromasia was followed microscopically for up to 1 h on several sections of the skin of the operated and control rats coloured simultaneously.

In the control rats, metachromasia of the derma was present in all cases after 12 min; in 13 cases the metachromatic stain intensified reaching an optimum after 20 min; in the other 2 cases, the optimum was reached after 30 min.

Of the operated rats, in only 10 cases was metachromatic staining evident after 12 min and in the other 4 cases only after 20 min; an optimal coloration after 20 min was reached in 6 cases, while the remaining 8 cases only reached this stage after 40 min. These results show that metachromasia of the derma in the operated animals develops less easily than in the controls, indicating structural changes of the mucopolysaccharides. The minor differences can perhaps be explained by the different lengths of time since the operation, or by the presence of accessory parathyroids in some of these animals.

Because of the normal calcium level in the blood of the operated rats, we are inclined to believe that mucopolysaccharide changes, not only of the organic bone matrix but also of the other connective tissues, are primarily related to the parathyroid function¹¹.

Riassunto. Nei ratti femmine paratiroidectomizzati si osserva un aumento della permeabilità vasale ed una modificazione della colorazione metacromatica del derma, indipendenti dalla concentrazione del calcio nel sangue. Tali variazioni vengono attribuite a modificazioni strutturali dei mucopolisaccaridi della sostanza fondamentale del connettivo.

A. DEBENEDETTI, A. MILLO,
B. PIERINI, and L. RAMPICHINI

Facoltà di Veterinaria, Università di Perugia (Italy), September 20, 1962.

¹¹ This investigation was partly supported by a grant from the Consiglio Nazionale delle Ricerche.

Infectious RNA from Ranikhet Disease Virus and its Preservation with Lipid Treatment

Infectious RNA have been isolated from a number of simple plant and animal viruses by the phenol extraction procedure^{1,2}. We now report the isolation of infectious RNA, by this method, from Ranikhet disease virus³ (Indian strain of NDV), a virus belonging to the more complex Myxovirus group. Preliminary experiments indicated that the infectious viral RNA is extremely susceptible to its environment and, unlike the virus itself, loses its infectivity on dilution, variation in environment temperature, storage and contact with ribonuclease. The high lipid content of this virus (ca. 50%) led us to investigate any preservative action lipids might have on the RNA. It was found that treatment of the RNA, immediately after isolation, with viral or mouse brain lipids, resulted in the RNA being able to retain its infectivity on dilution and even on treatment with ribonuclease.

The virus material used in the first series of the present experiments was freshly harvested allantoic fluid from chick-embryos infected with Ranikhet disease virus, clarified by centrifugation at 1500 rpm. The need to establish the identity of the isolated RNA necessitated the use of purified virus. This was obtained by adsorption of the virus from infective allantoic fluid on to aluminium phosphate gel at pH 6 at 0°C, elution at pH 8 at 37°C and isolation by ultracentrifugation at 40000 rpm for 2 1/2 h (Miller and Schlesinger procedure⁴ modified by O.P.B. and Dr. Nitya Nand). The purified virus was then resuspended in M/25 phosphate buffer pH 6. The isolation of

¹ A. GIERER and G. SCHRAMM, *Z. Naturforsch.* 11b, 138 (1956).

² J. J. HOLLAND, B. H. HOVER, L. C. McLAREN, and J. T. SYVERTON, *J. exp. Med.* 112, 821 (1960).

³ O. P. BABBAR and M. M. DHAR, *J. sci. industr. Res. (India)* 15C, 249 (1956).

⁴ R. C. VALENTINE and A. ISAACS, *J. gen. Microbiol.* 16, 680 (1957).