synthesis, so that at the time vitamin K was administered, a full response could not be obtained To test for this possibility, actinomycin (140 μg/100 g) was given i v. 4 h before the animals were killed. As shown in Figure 1, d, liver slices from such animals responded normally to vitamin K. OLSON also measured the change in plasma clotting factors 6 h after the i.p. injection of vitamin K. It has been found previously that when vitamin K is solubilized with Tween 80 and given i.v. to anticoagulant-treated animals, an increase of the plasma concentration of clotting factors can be detected already after 30 min and can be monitored in the same animal by the taking of blood samples at frequent intervals[11]. When actinomycin (350 μg/100 g) was given i.v. by injection into a tail vein 2 h before vitamin K_1 (50 μg/100 g) the rate of increase of the plasma levels of factor VII during the next 2 h was reduced only slightly (Figure 2). However,

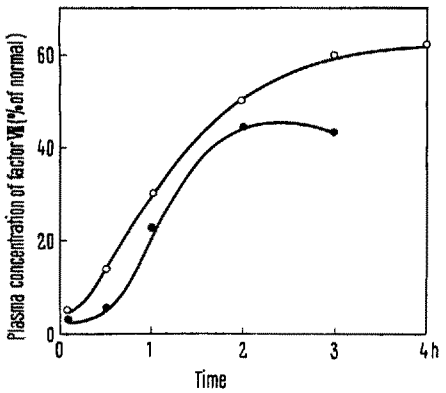

Fig. 2. Effect of actinomycin on the increase of the plasma concentration of factor VII by vitamin K_1 in anticoagulant-treated rats. O—O vitamin K_1 (50 μg/100 g), ●—● actinomycin (350 μg/100 g) given 2 h before vitamin K_1 (50 μg/100 g). Confirmatory results were obtained in 2 additional experiments.

the animals that had received actinomycin were definitely in a poor state and it became progressively more and more difficult to get blood samples by tail vein puncture. While the animals that had received actinomycin died within 24 h, in the control animals factor VII concentrations had returned to normal after 24 h.

The failure to demonstrate an effect by actinomycin in vitro and the likelihood that actinomycin can produce non-specific toxic effects under the conditions where inhibition has been reported in intact animals rules out the possibility that vitamin K acts at the level of mRNA synthesis. The present findings, therefore, do not support the hypothesis that vitamin K, and by analogy other fat-soluble vitamins, operate to control the synthesis of specific proteins by regulating mRNA formation[12,13].

Zusammenfassung. Lebergewebeschnitte mit Cumarin antikoagulentienvorbehandelter Ratten zeigen den Faktor VII im Medium nur beim Zusatz von Vitamin K. Die Vitamin-K-Wirkung wird bei gleichzeitigem Zusatz von Aktinomycin D nicht beeinflusst. Diese Beobachtung stimmt nicht überein mit der Hypothese, dass Vitamin K durch Beförderung der Auslösung der für die Synthese des Faktors VII spezifischen t-RNA eine Wirkung ausübt.

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The Effect of Basic Vital Dyes on the Acid Phosphatase Activity of the Granulated Juxtaglomerular Cells

The vital staining of the granulated juxtaglomerular cells (JGC) with neutral red was observed for the first time by SUGIYAMA[1], later HARADA[2], WORTHINGTON[3] and CORBASCIO[4] studied the details of the staining mechanism. ROSENBAUER[5] and SZOKOLY, GOMBA and SOLTÉSZ[6] found some other basic dyes staining the JGC vitally in addition to the neutral red. According to RUYTER'S[7] and our (GOMBA, SOLTÉSZ and SZOKOLY[8]) histochemical investigations the JGC of the mouse kidney contains acid phosphatase localized most probably to the secretory granules. Because, in the case of the neutral red, the vital staining properties of some intracytoplasmic granules has been found by HAFIEK and KOVÁCS[9] to be related with their acid phosphatase activity, therefore we examined the effect of some basic vital dyes on the acid phosphatase activity of the mouse JGC.

80 white mice of our own strain, of both sexes, weighing about 25 g, were used. The vital stains were the following: neutral red, neutral violet, acridin orange, Nile blue sulphate, brilliant cresyl blue. The dyes were dissolved in 1–2% concentrations in 0.85% NaCl. 1–2 ml of these solutions was injected into the tail vein or into the thoracic aorta (through the cranially ligated left common carotid artery) and the animals were killed 2–3 min after the injection. Each stain was tested also giving 1 ml 1% solution i.p. In those cases we killed the mice after 15, 30, 60 min or 6 and 8 h. In some cases the neutral red, Nile blue

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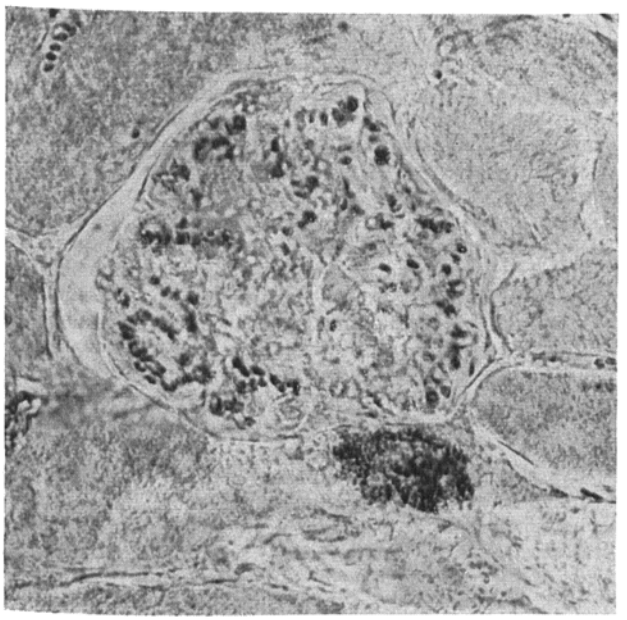

Fig. 1. The granulated juxtaglomerular cells are vitalstained with neutral red. Freeze-dried cryostat section. × 400.

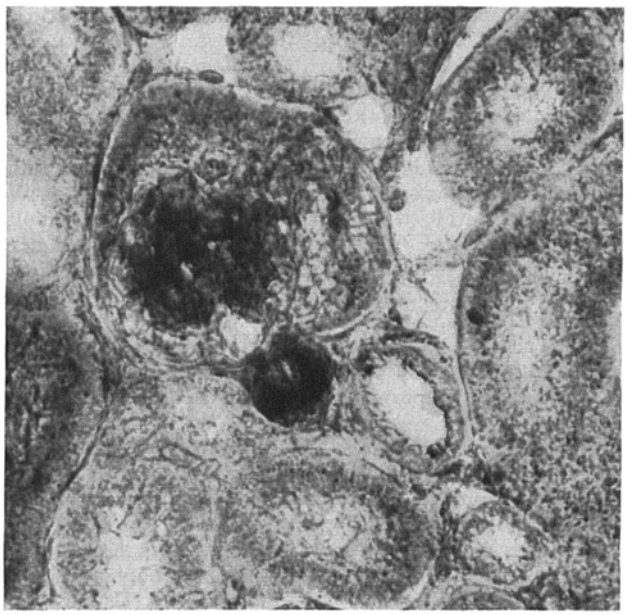

Fig. 2. Positive acid phosphatase reaction in the granulated juxtaglomerular cells. The glomerulum is also positive. GÖMÖRI's lead method modified by BARKA. × 400.

sulphate and brilliant cresyl blue was injected directly into the renal parenchym of the living animal according to ROSENBAUER's[5] method and the kidneys were immediately removed.

The removed kidneys were cut in half and fixed at 4 °C for 16 h in 10% Ca formol adjusted to pH 7 with 0.1 *N* NaOH. The sections prepared on a freezing microtome were incubated for 60, 90, 120 min for acid phosphatase according to GÖMÖRI's lead method modified by BARKA[10].

The existence of the vital staining was controlled partly in freeze-dryed cryostate sections, partly in squash preparates made from thin fresh kidney slices[5]. Before freeze-drying thin pieces of fresh kidney tissue were quenched in isopentane cooled with carbon dioxide snow and the cryostate sections prepared, were dried in a closed vessel over phosphorpentoxyde at – 20 °C under atmospheric pressure for 24 h. The dried sections were put on microscopic slides, wet with bensol and mounted in Canada balsam.

In the animals injected i.p. with neutral red, the acid phosphatase activity of the JGC disappeared in 15 min and returned only 6 h after the injection. After 1 h, the acid phosphatase positive granules (lysosomes) of the tubular cells lost their activity too, but the reaction of the glomeruli persisted. After the i.p. injection of the other dyes – although they vital-stained the JGC granules – the normal activity of the JGC, tubular lysosomes and glomeruli did not change, sometimes 1 h after the injection a mild weakening of the reaction was seen, but often it was rather moderately increased in the structures mentioned.

After intravenous, intraarterial and intrarenal injection the result was the same: the neutral red immediately abolished the enzyme activity while the other dyes did not.

The explanation of our observations is not clear. It is possible that the neutral red was combined with the enzyme, or – as KOENIG[11] wrote – with the structural phosphatides of the membrane or matrix of the granules, and interfered with the activity of the enzyme molecules. It is interesting to note that during the formol fixation the dye was extracted from the granules, that is, at the time of the histochemical reaction it was not present – or at least not light microscopically visible. Therefore the other possibility is that the enzyme was 'exhausted' during the cellular process of dye intake or its resynthesis was inhibited by the stain. We shall probably be able to get nearer this problem by investigating the neutral red – acid phosphatase interaction in tissue extracts.

We cannot conclude why only the neutral red abolished the acid phosphatase activity; this needs further investigation.

The inhibition of the acid phosphatase activity of the JGC by the neutral red offers a possibility to investigate the role of this enzyme in the secretory mechanism of these cells.

Zusammenfassung. Die histochemisch nachweisbare saure Phosphataseaktivität der juxtaglomerulären granulierten Zellen wurde durch Vitalfärbung mit Neutralrot aufgehoben. Diese Zellen sind auch mit anderen Vitalfarbstoffen: Acridinorange, Nilblausulfat, Brillantkresylblau färbbar, ohne dass die saure Phosphataseaktivität aufzuheben ist.

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