the enlargement begins rather abruptly on the side of the 'small intestine', but gradually diminishes toward the rectum. It shows circular contraction waves, running briskly in a peristaltic or even antiperistaltic direction. There is (Figure 2) a flap-like valve with sphincter, similar to that found in the adult forms and seemingly homologous to the ileo-caecal valve of higher vertebrates. The flow of the contents, rich with water and mucus, pushed from the small into the enlarged intestine, is often clearly visible: the main flow streams centrally, whereas the flow close to the walls is slowed down and probably reversed and the liquid is disimbibed. This kind of 'turbulence' can probably explain why colonies of opalinids and microorganisms are characteristically located in a tract of the lumen near the wall, at a short distance from the valve. Perhaps the presence of this localized reservoir of parasites and commensals corresponds, functionally at least, to some primitive form of the caecum.

Riassunto. Nell'intestino posteriore dei girini alberga una flora microbica stabile, simile a quella degli Anfibi adulti. Ciò ben si accorda col reperto di peculiari aspetti morfo-funzionali dell'intestino posteriore dei girini, somiglianti a quelli di un vero e proprio colon.

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Evidence for the Precipitating Activity of Insulin Antibodies

Today there is no doubt about the antigenicity of insulin. On the other hand, conflicting ideas exist about the antigenic character of the insulin molecule, since it has frequently been reported that insulin does not precipitate[1-3]. Above all, BERSON and YALOW[4,5] concluded that, in its reaction with antibodies in human antisera, insulin behaves as if it were univalent. This conclusion was drawn from studies on the sedimentation velocity of insulin-antibody complexes in the region of marked antibody excess, which showed that only a single antibody molecule combined with insulin. In contrast, however, precipitating antibodies to insulin have been obtained in the last 7 years in man, horse, guinea-pig, sheep and rabbit[6]. Finally, the study of ARQUILLA and FINN[7] presents evidence that the insulin molecule has more than one antigenic determinant site. Considering the frequently strong positive results obtained with the Boyden technique, neutralization tests, and complement fixation reactions[8], there is in principle no reason for assuming that insulin antibodies do not precipitate. Unfortunately, however, none of the reports about the precipitating activity of insulin antibodies affords a direct demonstration for such an activity, since it has been shown that many preparations of insulin recrystallized a number of times contain antigenic impurities showing no species specificity[9]. Our results, reported here in brief, give evidence for the precipitating activity of antibodies against insulin.

A group of 20 guinea-pigs was immunized with crystalline pig-insulin (free from glucagon; zinc content 0.29%), obtained from Farbwerke Hoechst. The animals received at weekly intervals a total of 10 injections of 1 mg insulin incorporated in complete Freund's adjuvant. The first 4 injections were given i.m., the next 4 doses s.c., the 9th i.c. and the 10th into the footpad. Shortly before and also 4, 8 and 12 h after the first 4 injections, each guinea-pig received a 6 ml i.p. injection consisting of 5% glucose. The animals were bled 10 days after the last injection. Ten guinea-pigs were likewise immunized with oxidized pig-insulin and 15 guinea-pigs with photooxidized pig-insulin. In the latter case, 6 of the 15 animals received with each injection 3 mg of the photooxidized preparation. The oxidative separation of pig-insulin into the sulphonates of the A- and B-chains was performed as described[10]. Test for the completeness of the separation was performed by means of paper electrophoresis, using 0.05 *M* veronal buffer (pH 7.4).

Photooxidized pig-insulin was prepared employing the photooxidation amino acid disruption method, using molecular oxygen in the presence of methylene blue as catalyst[11] as described elsewhere[12]. This procedure results in the preferential disruption of the imidazole ring, while tryptophane, tyrosine, methionine and cystine are essentially less involved and in decreasing degree. Photooxidation of insulin results in a complete loss of hormonal activity if all the imidazole rings are opened[13,14] even without changes in the tertiary structure of the insulin molecule[14]. The preparation obtained by us had a tyrosine content of 68%, while histidine was diminished to less than 5%, as determined photometrically[13].

The antisera produced against the various insulin preparations were investigated by the agar gel precipitation method, capillary precipitation and immunoelectrophoretic analysis.

Precipitating insulin antibodies could be found in antisera of animals immunized with either native or photo-

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oxidized pig-insulin. Both types of sera react with native as well as with photooxidized pig-insulin, with a second sample of crystalline pig-insulin (Novo) and with 2 different samples of repeatedly recrystallized ox-insulin (Farbwerke Hoechst and Novo). On the other hand, antisera either against native or photooxidized pig-insulin did not react with oxidized pig-insulin or with single or mixed A- and B-chains from ox-insulin prepared by the method of sulphitolysis[15,16]. Finally, the precipitating activity of antisera to native and photooxidized pig-insulin could be completely removed by addition of native or photooxidized pig-insulin. No precipitation line appeared after immunoelectrophoretic analysis of the supernatants of such absorbed antisera.

On the other hand, no precipitating insulin antibodies could be demonstrated in the sera of guinea-pigs immunized with the oxidized pig-insulin preparation. The loss of immunogenicity in this preparation may be related, at least partly, to the fact that the oxidation procedure results in a reduction of the sedimentation coefficient to 1,53 *S*, compared with 3,2 *S* and 3,3 *S*, respectively, for native and photooxidized pig-insulin, as found in sedimentation studies using analytical ultracentrifugation methods.

Should the observed precipitins be directed to antigenic impurities possibly present in repeatedly recrystallized insulin preparations, then one would expect these antibodies to react with similar impurities present in the oxidized insulin preparation. That the demonstrated antibodies are really directed to the insulin can be seen by the fact that in immunoelectrophoretic studies the precipitation lines formed with photooxidized pig-insulin were always localized much more towards the anode than those formed by the reaction of antisera to native or photooxidized pig-insulin with native pig- or ox-insulin. This means that photooxidized insulin has a higher electrophoretic mobility than the native preparation. This phenomenon can be explained by the observation[13] that photooxidized insulin in comparison with native insulin shows an isoelectric precipitation range which is shifted about 1.5 pH units towards the acid side. The results presented here may allow one to conclude that insulin has more than one antigenic determinant site.

Zusammenfassung. Die serologische Untersuchung von gegen natives und photooxidiertes Schweineinsulin gerichteten Meerschweinchenantiseren führte zu dem Ergebnis, dass die Antikörper gegen Insulin gerichtet sind, nicht aber gegen möglicherweise in den kristallinen Insulinpräparationen vorhandene antigene Verunreinigungen.

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Production of Antibodies Against Synthetic Angiotensin II in Various Animal Species

Circulating antibodies to angiotensin II have been produced in the rabbit by injection of benzoylangiotensin-azo-bovine γ-globulin[1], by injection of conjugates containing albumin and the peptide bound covalently by means of carbodiimide reagents[2], and by immunization with synthetic branch-chain copolymers comprising backbones of poly-L-lysine and branches of angiotensin[3]. The in vitro immunochemical properties of anti-angiotensin have been determined by hapten inhibition tests using a quantitative micro-complement fixation[4] and, more recently, by means of a modified ammonium sulphate precipitation technique[5]. Some evidence has been found for immunization against angiotensin-amide in the rat after treatment with an angiotensin-II-carbodiimide-rat albumin complex[6]. In the present investigation an attempt was made to immunize animals from several species against angiotensin-amide.

Asp(NH_2)[1]-Val[5]-angiotensin-II (AII) was conjugated to bovine γ-globulin (BGG) or to human γ-globulin (HGG) by means of carbodiimide (CDI) condensation according to the procedure of GOODFRIEND et al.[2]. This antigen was given in complete Freund's adjuvant (Difco). Alum-precipitated AII ('coarse') was prepared as described earlier[7]. Rabbits (3–3.5 kg) were injected twice with 0.4 mg AII-CDI-BGG in multiple s.c. sites and once with the same amount of antigen in the foot pads. The injections were given at 1 month intervals. Rats (60–130 g initial weight) received 1–3 injections of 0.4–0.6 mg AII-protein conjugate s.c. or in the foot pads followed by an i.p. injection of 0.3 or 0.6 mg antigen 4–6 weeks later. Cats (3–5 kg) were injected at multiple s.c. sites with a total amount of 1.6 mg AII-CDI-BGG. Two mongrel dogs (20 and 21 kg) received 3 injections of AII-CDI-BGG. Over a period of 18 weeks a total amount of 22 mg was administered either i.m. or s.c. One month after the last injection of the emulsion 4 i.v. injections of 40 mg alum-precipitated AII were given every second week. Anti-angiotensin activity was determined 10–21 days after the final antigen injection in the rabbit and the rat and 6 weeks after the last antigen injection in the cat. The dogs were bled at intervals during the whole immunization period, as well as 4 weeks after the last injection in order to analyze small samples of serum for anti-AII activity. The pressor

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