lowing the infection. A decrease in serum albumin and an increase in α_2-globulin occurred 6-7 days after infection. More pronounced changes were seen in the glycoproteins. The "glycoalbumin" showed a 34% decrease as compared to 5% for serum albumin. "α_1-glycoprotein" had a significant increase on day 5. Both the "glycoalbumin" and "α_1-glycoprotein" were almost at an 0.05 level of significance by day 3 post exposure, prior to the onset of fever, and reached a significant level of 0.001 on the day of maximum fever.

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Detection of a Kinin Substance in the Testis of Guinea Pigs After Induced Allergic Orchitis.* (31450)

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It is generally assumed that in heterologous induced allergic processes liberation or activation of certain amines and/or polypeptides (plasmakinins) apparently implicated in the development of inflammatory signs and anaphylactic symptoms takes place(1,2).

Lack of data about the presence of similar substances in the male gonad of guinea pigs, carrying an induced allergic orchitis(3,4) prompted us to investigate the problem in these homo- or autosensitized animals. Correlation between the appearance of these substances and the development of testicular lesions and of circulating antispermatic antibodies was also attempted.

Material and methods. A total of 104 healthy adult male guinea pigs weighing 400 to 500 g divided into 4 groups was used. Group I comprised normal control animals. Group II included animals intradermally sensitized in the dorsum with one dose of the antigen consisting of homologous testicular homogenate plus complete Freund adjuvant (Difco 0638), as recommended by Freund *et al*(3). Groups III and IV also used as controls were injected with testicular homogenate or with adjuvant alone in doses similar to those administered in Group II (Table I). After sensitization animals from all groups were killed at intervals of time between 3 and 40 days (Table II). Blood samples were taken by cardiac puncture and circulating antispermatic antibodies determined by complement fixing test (50% hemolysis), using the supernatant of testicular homogenate at an optimal dilution of 1/250. A portion of the gonad was fixed in Bouin's liquid for routine histological examination. Lesions were estimated as vascular changes, accumulation of mononuclear cells, cytolysis and sloughing of the germinative epithelium. The remainder of testicular tissue was used for detection of kinin substances. After dissecting out the albuginea, the mass of tubules and intertubular connective tissue structures were immediately homogenized in buffered saline at pH 7.5 for 5 minutes. Proteins were then precipitated by boiling in a water bath at 100°C for 3 minutes and, after centrifugation, 0.2 ml of the supernatant was submitted to standard biological tests to verify the existence of spasmogenic substances in the testicular tissue. For the biological tests 3 kinds of organ preparation were used: normal guinea pig ileum and rat duodenum, suspended in 2 ml Tyrode solution bath at 34°C and 30°C,

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TABLE I. Percentage of Animals Showing Release of Polypeptide, Testicular Lesions and Antibody in Induced Allergic Aspermatogenesis.

	Experimental groups	No. of animals	Concentration polypeptide in testicular tissue (μg/g)	% of animals showing: Polypeptide in testicular tissue	Testicular lesion	Circulating antibody
I	Normal, not sensitized	30	0	0	0	0
II	Testicular homogenate + complete adjuvant	49	.731 ± .013	85.7	65.3	38.8
III	Testicular homogenate alone	12	0	0	0	0
IV	Complete adjuvant alone	13	.030 ± .029	7.6	0	0

TABLE II. Correlation Between Amount of Polypeptide in Testicular Tissue and Percentage of Animals Showing Release of Polypeptide, Testicular Lesion and Circulating Antibody in Each Period After Sensitization.

Days after sensitization	No. of animals	Amt of polypeptide in testicular tissue (μg/g)		% of animals showing: Polypeptide in testicular tissue	Testicular lesion	Circulating antibody
3– 5	12	.38 ± .11	(p .05)	58.3	33.3	25.0
23–26	14	1.23 ± .21	(p .05)	100.0	78.6	50.0
12–15	12	1.03 ± .22	(p .05)	83.3	75.0	33.3
36–40	11	.40 ± .08		100.0	72.7	45.4

respectively, and rat uterus immersed in Jalon's solution at 30°C. The presence of smooth muscle stimulating substances was separately checked in the testicular supernatant of all specimens corresponding to the 4 groups of animals at different periods after sensitization. To exclude the eventual presence of histamine, acetylcholine or serotonin in the testicular extract, the corresponding antagonistic drug, *i.e.,* mepyramine, 0.2 μg/ml, atropine, 0.1 μg/ml, lysergic acid diethylamide or methylsergide both at 0.1 μg/ml were separately added to the medium in the biological tests. The probable polypeptide nature of the active substance was tested by prior incubation of the supernatant of testicular tissue with α-chymotrypsin 0.1 mg/ml for 15 minutes at 37°C. The amount of the active substance present was determined based on similar stimulating action provided by a standard preparation of bradykinin (B.R.S. 640, Sandoz) and estimated as μg per gram of wet tissue. The significance of the results obtained was statistically verified.

Results. A substance which produced a slow contraction of the smooth muscle of guinea pig ileum and rat uterus was detected in the testicular extracts after sensitization. Rat uterus was 10 times more sensitive than guinea pig ileum to this substance, this being considered a characteristic of the biological action. The absence of counteracting effect upon the spasmogenic action, with the addition to the medium of the corresponding antagonistic agents, made the results not attributable to histamine, acetylcholine or serotonin.

On the other hand a typical relaxation obtained upon the rat duodenum is very similar to the one induced by bradykinin. In addition the effect on the guinea pig ileum disappeared when prior incubation with α-chymotrypsin was performed. These findings suggest that a substance of polypeptide nature resembling bradykinin appears responsible for the spasmogenic action. As seen in Table I, a high percentage of completely sensitized animals of Group II gave positive results (85.7%) in contrast to the negative results of Group I, unsensitized animals, and of Group III, sensitized with antigen alone. One of 13 animals of Group IV sensitized with adjuvant alone, showed a detectable amount of stimulating substance. Table I also shows a better correlation in the same animals, between the percentage of those with detectable active

substance (85.7%) and with lesions in the gonads (65.3%) than with those showing antispermatic antibodies (38.8%). In these cases the antibodies demonstrated by fixing complement test were positive between 1/100 to 1/400.

Table II shows chronologically that the active substance appeared from the first days after sensitization and increased significantly up to 12-15 days (1.03 ± 0.22 μg/g). Thereafter no significant change was found until 23-26 days (1.23 ± 0.21 μg/g), which is in contrast to a significant decrease detectable at 36-40 days (0.40 ± 0.08). The same Table also shows a close correlation between the progressive percentage of animals with detectable active substance and testicular lesions, which reach a peak between 23-40 days after sensitization. This correlation becomes less evident when considering the incidence of circulating antibody in the same periods. Lesions of the gonads were revealed as congestion and edema of intertubular spaces accompanied by a moderate mononuclear cell infiltration observable during the first weeks. Seminiferous tubule alteration, consisting of sloughing and germinal cell vacuolization, was an incipient sign during the same periods, but predominated after the second week. Complete tubular damage was evident between 30 to 40 days. No lesion was apparent in the interstitial Leydig cells or other connective tissue cells, such as fibroblasts or mast cells.

Discussion. The evidence presented strongly suggests that, under the biological methods and conditions used, a smooth muscle spasmogenic substance appears in the testicular tissue of guinea pigs after sensitization with testicular homogenate plus Freund adjuvant. This assumption is reinforced by the fact that normal or incompletely sensitized animals used as controls gave negative or negligible results.

The increasing concentration of the spasmogenic substance in the testis after sensitization, and the progressive percentage of animals showing this substance and testicular lesions favor the interpretation of some connection between both phenomena. The same correlation with the antispermatic antibodies is not so clear, probably due to the often low or undetectable level and transient character of these homo- or auto-antibodies in allergic orchitis(3,4,5). Vascular changes, *i.e.*, congestion and serous edema, accompanied by incipient germinal cell damage, constitute the visible testicular lesions during the first weeks, followed by destruction of germinative epithelium. Consequently, the phase of vascular changes and the initiation of germinal cell damage appear more related to the presence of the spasmogenic substance. The nature of this substance, which seems to be a polypeptide, probably related to or similar to bradykinin, is deduced from its biological activity upon smooth muscle and the lytic effect of chymotrypsin.

In this respect, several peptides like bradykinin and related substances have been found in different tissues, and their action is sufficiently potent to suggest that their function might result in vasodilatation, increased permeability and migration of blood cells(6,7,8). Some reports claim that proteolytic enzymes are activated during tissue destruction in inflammation(9,10), but there was no conclusive demonstration that the action of these hydrolases leads to the formation of different types of kinins. It is interesting to note that in guinea pigs sensitized similarly to those used in our experiments, the lungs release histamine when this tissue is incubated with fresh spermatozoa(11). Further study of the immunological system of allergic orchitis is needed to clarify the plasma-kinin site and forming mechanism, its dependence of antigen-antibody interaction and its participation in the early vascular changes and germinal cell cytolysis.

Summary. In order to demonstrate the presence of amines or polypeptides (kinin substances) in the testicular tissue of adult guinea pigs during the development of an homologous allergic orchitis, the animals were sensitized with homologous testicular extract plus Freund adjuvant and studied at different periods after immunization. Standard biological tests using guinea pig ileum and rat uterus and duodenum were applied. It was observed that: 1) Histamine acetylcholine and serotonin were not detectable in the testicular tissue of

sensitized animals. 2) A polypeptide substance most probably related to bradykinin was present in a higher amount during the first weeks and began to disappear between 4 to 6 weeks. 3) There was a closer correlation between the appearance of this substance and testicular inflammation than with the development of antispermatic antibodies.

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Immunoassay of Human Insulin and Growth Hormone Simultaneously Using I-131 and I-125 Tracers.* (31451)

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The successful application of the two-antibody technique for assay of insulin(1) or growth hormone(2) has prompted an attempt at simultaneous immunoassay of these 2 hormones. The interrelationship of these 2 hormones in metabolic disorders has long been of interest(3,4,5).

Materials and methods. Each of 12 guinea pigs was injected subcutaneously with 1 mg of human growth hormone† (HGH) on days 0, 7, 14, 21, and 28. The HGH was prepared in a solution of 0.15 *M* NaCl, phosphate buffer (pH 7.4); the final concentration was 1 mg/ml. Equal volumes of HGH solution and lanolin-oil were mixed to a thick emulsion (6). After the 5 injections, each animal was bled *via* cardiac puncture on days 35 and 49, *i.e.*, at 7 and 21 days after the fifth injection. Three of the 12 guinea pigs responded with anti-human growth hormone serum (AHGHS-GP) suitable for immunoassay.

The anti-insulin guinea pig serum (AIS-GP) used in these experiments was prepared as previously described(7).

The I-125-HGH was prepared commercially.‡ I-131-insulin, porcine, was obtained from the same commercial source. The specific activity of each radioisotope labeled hormone was in excess of 100 mc/mg.

Anti-guinea pig serum (AGPS-R) was obtained from rabbits as previously described (7).

For immunoassay standards of HGH, the Wilhelmi preparation (NIH-HS-612B) was used. This was the same material sent to Abbott for labeling with I-125.

The insulin standards were a human insulin preparation (lot 515-734B-33) supplied by Dr. Mary Root of Eli Lilly Co. This lot of insulin had 23.4 units of activity per milligram.

The diluent used consisted of 1% bovine

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‡ Abbott Laboratories, North Chicago, Ill. This human growth hormone was kindly supplied by Dr. A. E. Wilhelmi, Emory Univ., Atlanta, Ga.