AN IMMUNOLOGIC STUDY OF THE LACTOGENIC HORMONE*

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Biological methods for the determination of lactogenic hormone are based chiefly on its action in inducing proliferation; they are effective in revealing comparatively large amounts of hormone (preparation injected systematically) [5], and are effective only for highly purified preparations (local injection), and in addition they require special strains of mice [6]. To determine the lactogenic hormone from its luteotropic activity requires hypophysectomized animals and is very laborious.

The application of precise and relatively simple immunological methods is of considerable interest. However, convenience and practicability are not the most important aspects of immunological studies. It is more significant that these methods can contribute further evidence of whether qualitative disturbances of hormone formation have occurred [1].

Unitl now no systematic immunological studies of the lactogenic hormone have been made which would allow the method to be tested. A number of problems had to be solved.

The Development of Immune Sera. It is known that the lactogenic hormone may induce the formation of specific antibodies [2, 3]. We have carried out experiments on the immunization of rabbits by use of bovine or sheep lactogenic hormone produced by the Leningrad Meat Combine (3 I.U./mg). Intramuscular injections of 20 mg/ml were given weekly for 3-4 weeks. The sera obtained reacted with the original preparations in reactions involving complement and precipitation in a gel, and also in Boiden's reaction. With precipitation in agar the hormones formed a single precipitation band with the corresponding sera.

Specificity of the Antilactogenic Immune Sera. The specificity of the sera obtained was tested by the complement-binding reaction with sera of large-horned cattle and pigs and with acid extracts of muscles of the corresponding animals. In all cases we obtained a negative result (Table 1). Many of the protein hormones were also tested in a complement-binding reaction with sera immune to the lactogenic hormone. As can be seen from Table 1, the hypophyseal gonadotropins of the sheep and pig and the serum gonadotropins of a mare in foal, and choriogonin gave negative results. However bovine somatotropin and high concentrations of ACTH (500 times higher than the sensitivity of the method for the determination of the lactogenic hormone) gave a positive reaction. These preparations reacted only in high concentrations, and we may therefore suppose that ACTH and somatotropin were not sufficiently free from lactogenic hormone.

Sensitivity of Immunological Reactions. The complement-binding reaction (as modified by V. I. Ioffe – prolonged binding at 4° with three doses of complement) enabled 0.1-0.5 μg of the preparation per ml to be determined. Recent studies [7] have shown that determination of the lactogenic hormone in Boiden's reaction caused only a very small increase in sensitivity when sera immune to sheep lactogenic hormone were used (up to 0.026 μg).

Species Specificity of the Lactogenic Hormone. Whether the immune sera obtained could be used for determination of the lactogenic hormone in man depends on whether the lactogenic hormones of different origins are species-specific, i.e., whether for each species a special serum immune to the corresponding lactogenic hormone is

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Table 1. Determination of the Lactogenic Hormone in the Complement-Binding Reaction by Means of Sera Immune to Sheep (Cow) Lactogenic Hormone

Preparation, and its initial concentration	Dilution of the preparation		
	1 : 10	1 : 100	1 : 1000
Sheep lactogenic hormone Bl mg/ml	+	+	+
Cow lactogenic hormone 1 mg/ml	+	+	+
Pig serum	–	–	–
Extract of sheep serum 6.25 g/ml	–	–	–
Cow serum	–	–	–
Extract of cow muscle 6.25 g/ml	–	–	–
Cow growth hormone 1 mg/ml	+	–	–
Pig and sheep gonadotropic hormones, 0.1 mg/ml	–	–	–
Gonadotropin from the serum of a mare in foal	–	–	–
Cow ACTH, 1 mg/ml	+	–	–
Choriogonin, 100 units	–	–	–

Table 2. Reaction of Sera to Sheep (Bovine) Lactogenic Hormone with Extracts of the Hypophyses of Various Species

Species of preparation	Dilution of preparation		
	1 : 10	1 : 100	1 : 1000
Bovine hypophyses*	+	+	+
Sheep hypophyses*	+	+	+
Human hypophyses*	–	–	–
Rabbit hypophyses	–	–	–
Rat hypophyses	–	–	–

*These extracts showed a varied lactogenic activity when tested by the increase of the crop of pigeons.

required or whether crossed reactions are possible. Previously it was pointed out that in the immunologic reactions cow and sheep lactogenic hormones always behaved in the same way although differences were observed with respect to neutralization of the biological action of the hormone by immune sera [3].

According to our results, sera immune to hormones from the cow hypophysis react equally with their own or sheep lactogenic hormones (Table 2).

A similar effect was obtained when the sera were tested against sheep lactogenic hormone, which rendered the serum completely inactive against the cow lactonegic hormone, and vice versa. All these effects were evidence of the identity of the two hormones in the complement-binding reaction. However, quite a different result was obtained when the test was made against extracts of human, rat, or rabbit hypophyses (Table 2).

The extracts were made with acid acetone [4]. The human hypophyseal extract showed considerable activity when tested in terms of the increase in the size of pigeon crop. However in the complement-binding reaction and the agar precipitation test extracts containing human lactogenic hormone failed to react with sera immune to sheep or cow lactogenic hormone. The same effect was obtained with respect to lactogenic hormones from rabbit or rat hypophyses. Extracts from sheep or bovine hypophyseal tissue prepared by a similar method reacted positively with the corresponding sera immune to lactogenic hormones.

Therefore human lactogenic hormone, like somatotropin is species specific. This result is interesting because, for example, we have been able to observe various degrees of crossed reactions in gonadotropins of various origins. It is therefore essential to study the problem of how to obtain purified lactogenic hormone from the human hypophysis in order to develop a specific immune serum. Similar results on the lactogenic hormone have been obtained recently by R. P. Levy, and Sampliner [8].

Our sera immune to cow and sheep prolactin were used to control the separation of hormones from the hypophysis and to develop a method for their determination in biological media prepared from tissues of these animals.

We may therefore draw the following conclusions:

1. Sera immune to cow or sheep lactogenic hormone do not react in the complement-binding reaction or in the agar precipitation test with extracts of human hypophysis having a marked lactogenic activity.

2. A species specificity of the lactogenic hormone towards these sera was observed also in a study of extracts from rat and rabbit hypophyses.

3. The possibility of obtaining sera immune to lactogenic hormone, the high sensitivity of the immunologic reaction, and its specificity make it essential to investigate the possibility of obtaining lactogenic hormone from human hypophyses, in order to establish an immunological method for its determination.

4. The immunological test for the lactogenic hormone is extremely convenient for use in making preparations or for determining their purity, and should be further tested with a view to determining qualitative variations in hormone formation in various pathological processes.

SUMMARY

Sera developed to be immune to cow and sheep lactogenic hormones failed to react with extracts containing human lactogenic hormone in the complement-fixation and agar-precipitation reactions. The same species specificity for the lactogenic hormone was also found in relation to extracts obtained from rat and rabbit hypophyses. The possibility of obtaining sera immune to lactogenic hormone, the high sensitivity of the immunological test, and its specificity make it essential to obtain lactogenic hormone from human hypophyses in order to develop an immunological method for its determination. Immunological testing of the lactogenic hormone would be a very convenient method for use in manufacture, and for determination of the purity of hormonal preparations; it should be further investigated in order to ascertain the possibility of detecting variations in the quality of the hormone in various pathological processes.

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