CONTENT OF IMMUNOREACTIVE INSULIN IN THE BLOOD OF RATS DURING REGENERATION OF THE LIVER

B. N. Leibush

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Partial heptectomy in rats causes no changes in the insulin content in the blood determined by a radiommunological method. The cause of the increase in insulin-like activity of the blood observed under these conditions was therefore an increase in the metabolic effect of the insulin due to regeneration of the liver.

Determination of the insulin-like activity (ILA) of the blood serum by a diaphragmatic method in rats after partial hepatectomy revealed an increase which continued for 3 weeks and was due to the fraction suppressed by antibodies against insulin. This fact appeared to indicate an increased insulin content in the blood of the hepatectomized animals, but two circumstances compelled a different explanation. First, the serum of the hepatectomized rats was found to have the property of potentiating the action of insulin on muscle. This property of the serum enables the ILA to be increased even if the blood insulin level is normal, because ILA characterizes the effect of the hormone and not its content. Second, although the ILA was increased when the serum was tested with respect to the glucose consumption of the diaphragm, it was normal if the same serum was tested by a lipid method.

The blood insulin level of rats after partial hepatectomy is evidently unchanged and the increase in ILA in this case depends entirely on potentiation of the metabolic effect of the hormone relative to muscle tissue. The validity of this hypothesis can be demonstrated by determining the blood insulin level in hepatectomized rats by a radioimmunological method, for by virtue of its high specificity, such a method can be used to estimate the content of the hormone with very great accuracy. The results of such a determination are described in this paper.

EXPERIMENTAL METHOD

Wistar rats weighing 190-240 g were used. Partial hepatectomy was performed by the usual method [2]. Between the 3rd and 18th days after the operation the insulin content was determined in blood serum obtained from fasting rats by a radioimmunological method.

The method of Catt et al. [1] was used. It is based on the use of test tubes treated with antibodies for separating labeled hormone bound with antibodies and free labeled hormone. The method was described by its authors for growth hormone, but with some modifications it has also been found suitable for determining the insulin content. The method is based on the ability of certain polymers to adsorb immunoglobulins firmly on their surface (the "solid-phase" principle). Tubes made of such a polymer, with antibodies fixed on them, can be used to remove an antigen from a solution poured into them, and after the tube is emptied the antigen–antibody complex remains on its wall. If the solution contains only labeled insulin, the radioactivity of the empty tube will be maximal. If unlabeled (to be determined) insulin is added to the same solution, the radioactivity of the tube after emptying will be correspondingly lower, because the unlabeled hormone will competitively prevent fixation of the antibodies by the labeled hormone.

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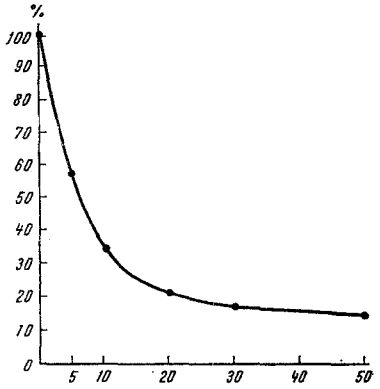

Fig. 1. Standard curve of insulin content. Abscissa, unlabeled insulin (in μl/ml); ordinate, adsorption of I^{125} on wall of tubes (tube containing labeled insulin only corresponds to 100%).

By using a series of known concentrations of unlabeled (crystalline) insulin a standard curve is plotted, and it gives the degree of decrease in radioactivity of the empty tube with an increase in the concentration of hormone to be determined. The radioactivity of the tube containing labeled insulin only was taken to be 100%.

Tubes made of polyethylene, 10 mm in diameter and with a capacity of 2 ml were used. Anti-insulin serum obtained in guinea pigs by the method of Wright and Norman [4] was diluted in the ratio of 1 : 1000 with 0.1 M sodium carbonate–bicarbonate buffer, pH 9.6, and added to the tubes in volumes of 1 ml. After standing for 3 h at room temperature the tubes were emptied, washed twice with 0.15 M NaCl solution (1 ml both times), and again once with buffer solution (2 ml). The treated tubes were kept at 4°C.

The insulin was labeled by the chloramine method of Hunter and Greenwood [3] with I^{125}, purified on a cellulose column as described by Yalow and Berson [5], and diluted with buffer solution. The buffer was made up from 0.02 M phosphate buffer, pH 7.4, to which human serum albumin (0.5%), NaCl (0.15 M), and merthiolate (0.002%) were added. Crystalline bovine insulin (USA) was used as the standard.

To a tube treated in this manner, 1 ml buffer solution containing either a standard quantity of insulin or the test serum in a dilution of 1 : 10 (each sample was tested in 3 tubes) and 0.2 ml labeled insulin were added in succession. The tube was incubated for 48 h at 4°C, then emptied and twice washed with cold water. Radioactivity of the empty tube was then measured on a type USD-1 scintillation counter. In the absence of unlabeled insulin, about 50% of added radioactivity was adsorbed on the wall of the tube. A typical standard curve is shown in Fig. 1. In the control tests, crystalline insulin added to the serum was revealed quantitatively, but serum diluted 3, 5, 10, and 20 times, with introduction of the appropriate factor, gave the same values (mean error 4.9%).

EXPERIMENTAL RESULTS

Tests were carried out on 14 intact and 14 hepatectomized rats. The normal content of immunoreactive insulin in the serum varied from 33 to 66 microunits (μu)/ml, with a mean value of 47 ± 3 μu/ml. Its level in the hepatectomized rats was 41 ± 3 μu/ml, with variations from 32 to 66 μu/ml. No significant difference was found between the two groups. The nepatectomized rats were grouped depending on the times after operation (0-7, 8-14, and 15-18 days). The insulin content at these times was 45 ± 2, 43 ± 7, and 33 ± 1 μu/ml respectively. After the 14th day, a small but statistically significant ($P < 0.02$) decrease in the insulin level was found.

Partial hepatectomy thus causes no significant changes in the content of immunoreactive insulin in the blood of rats and, in any event, it does not lead to its increase. Consequently, the prolonged and considerable (by almost 10 times) increase in "suppressible" ILA discovered by the diaphragmatic (biological) method after hepatectomy cannot be attributed to an increase in concentration of the hormone. The mechanism of potentiation of the insulin effect with respect to muscle tissue is thus solely responsible for this increase. Analysis of this mechanism is of considerable interest. Removal of part of the liver tissue is followed by intensive regeneration of the liver, and it is logical to suppose that as a result of this regenerative process a factor potentiating the action of insulin on muscle, and thus leading to an increased utilization of glucose by the muscle, appears in the blood of hepatectomized rats. Further investigations will be carried out to study the nature of this factor and to examine the biological role of the phenomenon described above.

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