Table 1.

Number of alpha particles hitting the target (in units of 10^{10} per cm^2)	Activity in units of declared I. U. per mg. of preparation
0	44 ± 6
0.8	31 ± 5
1.7	22 ± 4
2.4	13 ± 3
7.2	$< 7 \pm 3$
24	$< 4 \pm 2$

tion requires a knowledge of the shape of the HCG molecule. A maximum value can be obtained assuming a spherical molecule and a reasonable value for the effective radius of the alpha particle (2 AU). A molecular volume of about 150000 $(AU)^3$ is then obtained, and if a specific density of 1.3 is assumed as a reasonable value, this yields a maximum molecular weight of about 120000. The error in this figure may be +70 and —50 per cent.

SUMMARY

Using the toad spermiation test the inactivation cross section of human chorionic gonadotrophin for Po^{210} alpha particles was found to be $0.4 \cdot 10^{-10}$ cm^2. The probable error in this figure is +40 and —30 per cent.

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18, 293–298, 1955

From the Central Drug Research Institute, Lucknow, India (Dr. B. Mukerji)

STUDIES ON THE ORIGIN OF THE HYPERGLYCAEMIC GLYCOGENOLYTIC FACTOR (HGF)

By

M. R. Rajarama Rao and N. N. De

Soon after the discovery of insulin it was observed that most insulin preparations contained a fraction (*Gibbs et al.*, 1923) which had blood-sugar raising property. Later studies (*Sutherland & de Duve*, 1948) revealed that this substance (HGF) was present in the pancreas as well as in the gastric and duodenal mucosa of many species of animals. As HGF could be demonstrated in the extracts of pancreas which was fibrosed after ligature of the duct, and also in the extracts of pancreas in which the beta cells of the islets of Langerhans were selectively destroyed by alloxan, it was postulated that the alpha cells of the islet tissue might be the site of its formation. In support of this hypothesis *Vuylsteke et al.* (1952) reported a 60 % fall in HGF content of pancreas of guinea pigs in which the alpha cells were selectively destroyed by the administration of cobalt chloride. *Volk et al.* (1954), however, questioned the validity of this finding on the ground that cobalt chloride-treated canine pancreas contained normal amounts of HGF. In this connection *Sutherland & de Duve* (1948) suggested that the argentaphil cells may be the source of HGF in the gastric and duodenal mucosa. This view, again was contradicted by *Fodden* (1953) on the basis of morphological differences existing between the alpha cells and the argentaphil cells. Besides, *Larsson* (1951) reported that HGF could not be demonstrated in the extracts of the gastric mucosa of the hog even though the latter contained argentaphil cells.

The present study is a continuation of our previous work (*Rao & De*, 1954) and shows that the HGF is not produced by the alpha cells of the pancreatic islets, and that its presence could be demonstrated in the extracts of different organs which contain considerable quantities of connective or lymphatic tissues.

EXPERIMENTAL

Material and Methods. – Extraction and assay of HGF were based on the methods of *Sutherland & de Duve* (1948). Certain modifications were, however, introduced in the *in vitro* assay. Rabbit's liver slices weighing 50–60 mg. were incubated with HGF extracts at 37.5° C. in test tubes containing 4 ml. of chloride phosphate buffer pH 7.5). Oxygen was passed through the tubes for 40 minutes. The reaction was terminated by removing the liver slides and transferring an aliquot of the medium to test tubes containing 5 ml. of 0.45 % zinc sulphate solution and 1 ml. of 0.1 N sodium hydroxide. The tubes were placed in a boiling water bath for 3 minutes, and then filtered. Glucose in the filtrate was estimated by the method of *Hagedorn & Jensen* (1923). Generally five pairs of tubes were used; one for control, one for reference insulin (Insulin Lilly; ten units per ml. of the medium were found to produce maximal glucose output from the liver slices), and three pairs for three different samples or three different doses of the same sample of dialysed HGF extract. The results are expressed as per cent maximal effect produced by HGF extracts of different quantities of tissue tested according to the formula given by *Sutherland & de Duve* (1948).

In vivo tests were made on well-fed young rabbits. HGF extracts dissolved in saline were injected intravenously and blood drawn every five minutes for half an hour. Blood sugar was determined by the method of *Hagedorn & Jensen* (1923). Sections of pancreas from various groups of animals detailed below were examined histologically to ascertain the extent of damage caused by such agents as cobalt chloride. Small bits of pancreas were fixed in Bouin's fluid and the sections were stained by Gomori's chromic hematoxylin and phloxin stain. Frozen sections of formalin-fixed gastric mucosa were stained with silver (Fontana's fluid) by Masson's method.

Effect of cobaltisation on the HGF content of pancreas and gastric mucosa of rabbits

Fifteen healthy rabbits of either sex, weighing 1–1.5 kg. were selected for this study. Five were treated with cobalt chloride (50 mg./kg. body weight) by intravenous injection once a day for two days, and five with 25 mg./kg. body weight once a day for eight days. The remaining five rabbits were kept as control and received no treatment. All the fifteen rabbits were sacrificed on the termination of treatment and the pancreas and gastric mucosa collected separately for extraction and assay of HGF. The HGF contents of pancreas and gastric mucosa of cobaltised rabbits did not vary significantly from those of the untreated rabbits (Figs. 1 & 2). Histological studies, however, revealed that cobalt chloride had destroyed most of the alpha cells of the pancreatic islets, but did not have any effect on the argentaphil cells of the gastric mucosa.

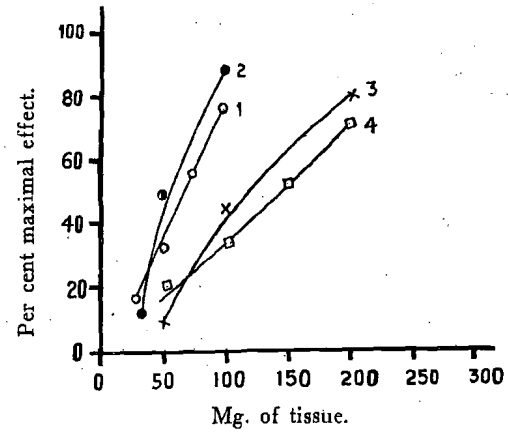

Fig. 1.
Assay curves for HGF (*in vitro*). 1) Normal rabbit pancreas. 2) Cobaltised rabbit pancreas. 3) Normal rabbit gastric mucosa. 4) Cobaltised rabbit gastric mucosa.

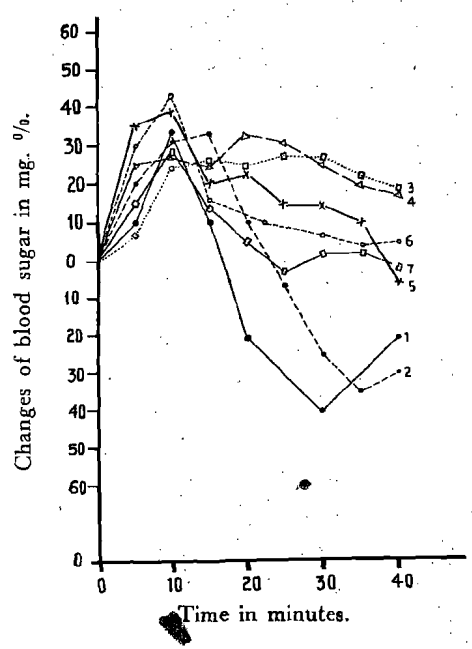

Fig. 2.
Assay curves for HGF (*in vivo*). 1) Normal rabbit pancreas (2 gm.). 2) Cobaltised rabbit pancreas (2 gm.). 3) Normal rabbit gastric mucosa (5 gm.). 4) Cobaltised rabbit gastric mucosa (5 gm.). 5) Alloxan diabetic rabbit pancreas (2 gm.). 6) Alloxan diabetic cobaltised rabbit pancreas (2 gm.). 7) Normal rabbit lymph nodes (4 gm.).

Effect of cobaltisation on the HGF content of the pancreas of alloxan diabetic rabbits

Ten healthy rabbits of either sex weighing 1–1.5 kg. were treated with alloxan monohydrate (150 mg./kg. body weight) by intravenous injection. Blood sugar determinations carried out after an interval of 3 days revealed that all the animals were severely diabetic, with blood sugar levels ranging from 500–700 mg./100 ml. Five of these diabetic rabbits were treated with cobalt chloride (50 mg./kg. body weight) intravenously once a day for two days. All the ten were sacrificed and the HGF contents of the pancreas measured. It was found that pancreas of both the groups contained normal amounts of HGF; *in vivo* tests revealed complete absence of insulin (Figs. 2 & 3).

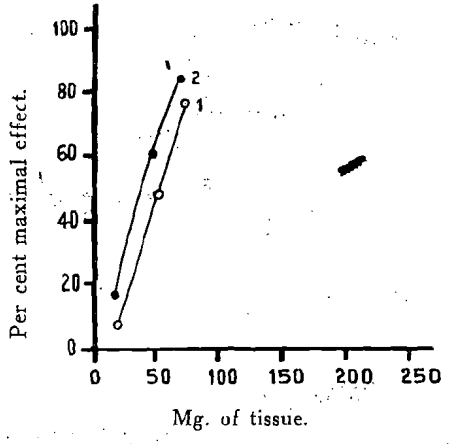

Fig. 3.
Assay curves for HGF (*in vitro*). 1) Alloxan diabetic rabbit pancreas. 2) Alloxan diabetic cobaltised rabbit pancreas.

It is inferred from the data that neither the alpha cells nor the beta cells of the islet tissue of the pancreas could be the source of HGF. It was considered probable that some other cells in the connective tissue stroma, such as the cells of lymphatic tissue often seen in the sections of the pancreas, or the connective tissue cells themselves might be the source of HGF. On this assumption it was thought possible to demonstrate the presence of this factor in other sites as well. Organs containing large amounts of lymphatic and connective tissues were, therefore, examined for HGF activity.

Distribution of HGF in other organs. – Abdominal lymph nodes, spleen, tongue, rectus muscle, and omentum from ten rabbits, skin from three dogs and three guinea pigs were extracted and assayed for HGF activity by methods similar to those employed for pancreas and gastric mucosa. Omentum and

muscle did not show any HGF activity. Lymph nodes, spleen, skin and tongue were found to contain appreciable quantities of HGF (Fig. 4).

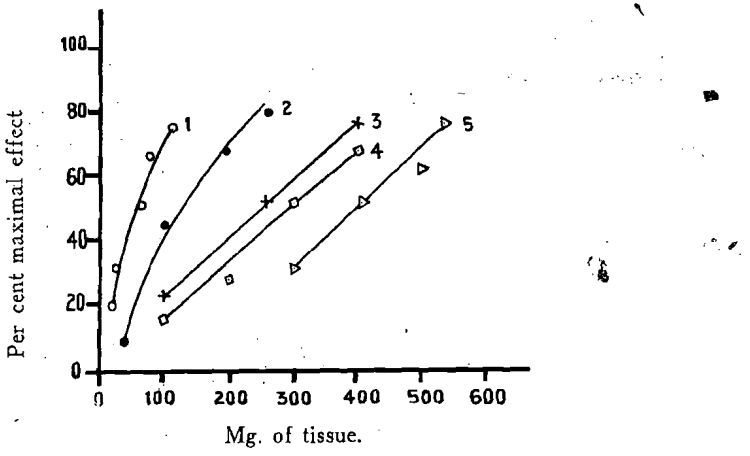

Fig. 4.
Assay curves for HGF (*in vitro*). 1) Normal rabbit's lymph glands. 2) Rabbits spleen. 3) Dog's skin. 4) Guinea pig's skin. 5) Rabbit's tongue.

DISCUSSION

From the excellent reviews of *Pincus* (1953), *de Duve* (1953) and *Volk et al.* (1954) on the nature of HGF one can make the general assumption that a factor (HGF) is present in extracts of pancreas and gastric mucosa and that it possesses blood sugar raising and glycogenolytic properties. The origin of HGF, its relation to carbohydrate metabolism and diabetes mellitus, and its hormonal nature are still matters of controversy. In spite of the fact that there are several chemical agents such as cobalt chloride, synthalin A, and diethylthiocarbonate which selectively destroy the pancreatic alpha cells, the origin of HGF from these cells has not been undisputably established. Though the techniques employed by *Volk et al.* (1954) for HGF assay are somewhat qualitative their results are significant and contradict the prevailing hypothesis that the alpha cells produce HGF. The data presented by *Volk et al.* (1954) and by the present authors on the pancreatic extracts of alloxan diabetic animals treated with cobalt chloride are of particular interest. Cobalt chloride is presumably as effective in destroying the alpha cells as alloxan is in destroying the beta cells. However, *in vivo* studies on extracts of pancreas thus treated show that while HGF activity is undiminished, insulin activity may be completely abolished. It appears, therefore, that the alpha cells are not the site of origin of HGF.

There is no direct experimental evidence in favour of the hypothesis that the gastric argentaphil cells produce HGF. *Larsson*'s (1951) observations on the hog's gastric mucosa appear to nullify such a hypothesis. *De Duve* (1953) suggested that there might be accessory islet tissue in the gastric mucosa acting as the source of HGF. If such accessory islet tissue in the gastric mucosa gave origin to HGF, it would be expected to yield an appreciable quantity of insulin as well. No hypoglycaemic effect of the extracts of gastric mucosa has, however, been reported so far.

The presence of HGF in lymph nodes, skin and other organs observed in the present study is an evidence which indicates that connective tissue or lymphatic tissue might be a source of HGF. The cellular elements of both of these tissues are present in the pancreas, gastric mucosa, and also in the other organs studied, and it is difficult at this stage, to specify which particular cell type is the source of HGF. Since the HGF extracts from these organs were prepared in the same way as the pancreatic extracts, and since it was also observed that HGF activity in these extracts was abolished by trypsin digestion, it is assumed that the substance responsible for hyperglycaemic glycogenolytic activity in the extracts of these organs is very similar to or perhaps identical with the HGF present in pancreatic extracts.

SUMMARY

1. Studies on the origin of HGF are presented in this paper.
2. Destruction of the alpha cells of the pancreatic islets with cobalt chloride is not attended with a fall in the HGF content of pancreatic extracts.
3. HGF activity is demonstrated in the extracts of lymph nodes, skin, tongue and spleen.
4. It is suggested that either the connective tissue or the lymphatic tissue is the source of HGF.

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THE INFLUENCE OF HYPERGLYCAEMIC GLYCOGENOLYTIC FACTOR (HGF) ON GLYCOGENOLYSIS IN SKIN

By

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In our earlier papers (*Rajarama Rao & De,* 1954, 1955) we have shown that the destruction of the alpha cells of the islet tissues of the pancreas is not associated with a fall in the HGF content of the pancreatic extract. We have further shown that HGF activity is demonstrable in the extracts of lymph nodes, skin, tongue and spleen. We have tentatively suggested that either the connective tissue or the lymphatic tissue is the source of HGF. Whether HGF could be considered a hormone, is as yet a matter of controversy. In order to clarify this point it was deemed necessary first to examine whether HGF exerted its action at the site of production or whether it was carried to a target of action distant from its point of origin. Since liver does not contain HGF but is the only known site of its action, it was generally believed that HGF was a hormone carried to the liver from the pancreas through the blood stream (*Sutherland,* 1951, *Foa et al.,* 1952). There is, however, no direct and conclusive evidence of the true hormonal nature of HGF; at best this can only be recognized as a possibility.

The extensive distribution of HGF in tissues suggests that it might exert its influence in the immediate neighbourhood of its formation. In the present paper, an attempt has been made to substantiate this hypothesis. Since skin contains phosphorylase and glycogen (*Shapiro & Werthiemer,* 1943) as well as HGF (*Rajarama Rao & De,* 1955), this tissue was chosen for a comparative study of the influence and site of action of HGF.

EXPERIMENTAL

Young rats (from the Institute colony) of either sex, weighing 60–80 gm., were used in this study. Skin from the back and sides was shaved and excised under

'seconal' anaesthesia and placed in chilled (0° C.) normal saline for 5–10 minutes. Small pieces of skin of approximately uniform size and weighing about 50 mg. were taken out, weighed and incubated with solutions of HGF in 2 ml. of phosphate-chloride buffer (pH 7.5) at 37° C. Oxygen was bubbled through the tubes for 10–45 minutes and the reaction was arrested by the addition of 5 ml. of 0.45 % $ZnSO_4$ and 1 ml. of 0.1 N NaOH. The tubes were then placed in boiling water bath for 3 minutes and the contents filtered. The filtrate was analysed for glucose output of skin pieces by the method of *Hagedorn & Jensen* (1923). Parallel experiments were carried out on liver slices weighing about 50 mg. Insulin (Lilly) containing appreciable quantities of HGF, dialysed against the buffer was employed as the source of HGF. Glucose output by skin or liver slices was calculated either as mg. of glucose formed per gm. of tissue or as per cent maximal output according to the formula of *Sutherland & de Duve* (1948).

RESULTS

Fig. 1 gives the time-course curve of glycogenolytic action of HGF in skin and liver slices. The glucose output is relatively small in the skin and stops after 15–20 minutes of incubation. In presence of HGF, the output of glucose increases by about 500 per cent during the first 15–20 minutes after which the reaction is arrested. The addition of second dose of the HGF, at this stage, has been found to promote the reaction for a similar period.

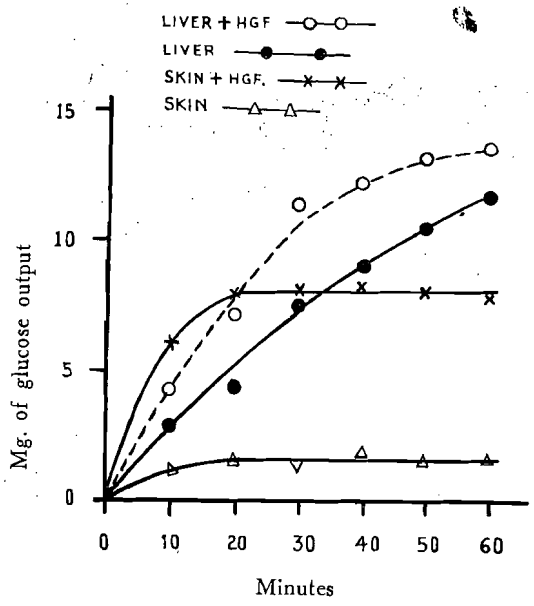

Fig. 1.
Time-course curves of glycogenolytic effect of insulin in skin and liver slices of rat. Insulin concentration was 30 units per ml. of the incubating medium.

Estimations of glycogen in the skin give a value which varies between 5–8 mg. of glycogen per gm. of material. After incubation with HGF, its glycogen content is reduced to 1–3 mg. per gm. In the case of the liver, the output of glucose, on the addition of HGF, amounts to an increase of 50–60 per cent.

Fig. 2 presents the effect of varying the concentration of insulin in the incubating medium, on the glucose output of skin and liver slices of equal weight. Higher concentrations of insulin were required for skin to produce maximal glucose output than for liver slices. Fig. 3 presents assay figures for HGF extracts of rat skin (HGF was extracted by the method of *Sutherland & de Duve*, 1948).

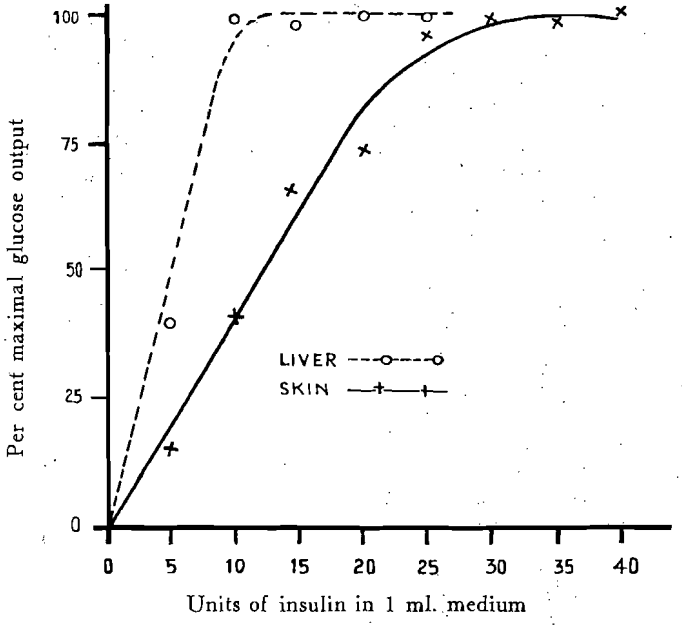

Fig. 2.
Effect of varying the concentration of insulin in the incubating medium on the glucose output of skin and liver slices of rat.

Table 1 shows that the addition of inorganic phosphate enhances the glucose output from skin while its effect on liver is relatively insignificant. The addition of phosphate increases the glycogenolytic action of HGF in both the skin and the liver slices. The addition of glucose-1-phosphate to the reaction mixture

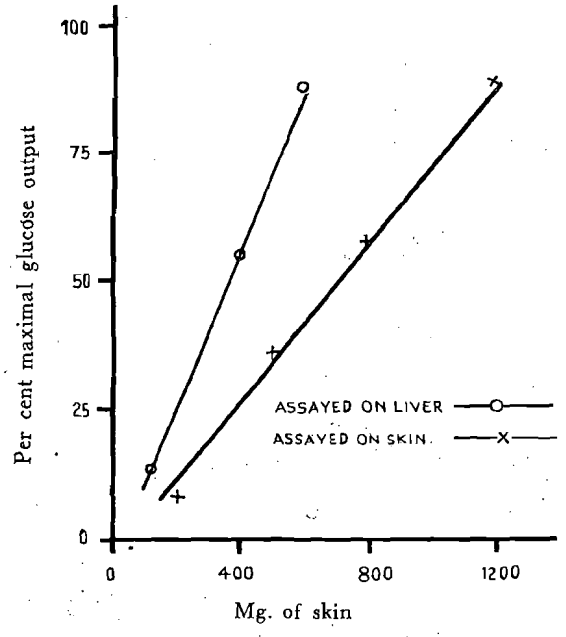

Fig. 3. In vitro assay of HGF extracted from the skin of rat.

Table 1.

Effect of inorganic phosphate, glucose-1-phosphate and insulin on the glucose output (mg.) in rat skin and liver slices.

Exp. no.	Additions per ml.	Skin		Liver	
		Glucose output per gm.	Glucose output per gm. minus control	Glucose output per gm.	Glucose output per gm. minus control
1.	None	0.28	–	6.84	–
	Insulin 20 units	2.12	1.84	9.17	2.33
	Phosphate 0.2 ml. 0.1 M	0.84	0.56	6.92	0.08
	Phosphate plus insulin 20 units	3.62	2.78	10.12	3.20
2.	None	0.92	–	5.21	–
	Insulin 20 units	4.88	3.96	8.43	3.22
	Glucose-1-phosphate 3 mg.	2.84	–	9.02	–
	Glucose-1-phosphate plus insulin 20 units	6.12	3.28	11.98	2.96

In experiment 1 chloride carbonate buffer was used, and in experiment 2 chloride phosphate buffer was used. Time of incubation, 30 minutes. Skin and liver slices weighed approximately 50 mg. each.

enhances the glucose output of both skin and liver slices showing thereby that skin, like liver, can dephosphorylate added sugar phosphate. Insulin had little effect on dephosphorylation of glucose-1-phosphate.

DISCUSSION

Unlike the liver the skin appears to be the site of both the origin and action of HGF. Whether this is true of *in vivo* conditions can be clarified only by further investigation. The epidermal and subcutaneous tissues which contain glycogen and the underlying connective tissues which secrete HGF are in close proximity, separated only by the tissue fluids. It would, therefore, appear that HGF is transported to its site of action in skin through the tissue fluids rather than through the blood stream. This possibility as well as the distribution of HGF at widely varying and integrally separate sites are considered as an indication of its localised formation and action, a feature unlike the characteristic functions of a typical hormone.

Our experiments support the earlier finding (*Sutherland & Cori*, 1948) that the point of action of HGF in the enzymatic reactions involved in glycogenolysis, is the phosphorylase system and that the skin phosphorylase is similar to that of liver, so far as its response to the action of HGF is concerned. The low glucose output of the control skin pieces and its 400–600 per cent acceleration in presence of HGF (Fig. 1) suggest that the skin phosphorylase is present mostly in an inactive form. Since the normal glucose output of skin pieces and its activation by HGF are quickly arrested (Fig. 1) it would appear that glycogenolysis and HGF activity are either inhibited or inactivated by a factor which remains to be elucidated. It is probably this factor associated with the skin that renders a significantly higher concentration of HGF necessary (cf. liver, Fig. 2) for maximal glucose output. This feature offers the possibility of using a wider range of doses in the multiple dose assay of HGF (Fig. 3). The skin, therefore, appears to offer a more suitable tissue for the *in vitro* assay of HGF, than liver.

SUMMARY

1. The influence of HGF on glycogenolysis in skin is reported. It has been found that the nature of action of HGF in skin is similar to that in liver.

2. Skin offers a wide range of response to the influence of HGF and, therefore, appears to be better suited experimental tissue for the *in vitro* assay of HGF than liver.

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Aus der Universitäts-Frauenklinik Kiel
(Professor Dr. med. E. Philipp)
und dem Pharmakologischen Institut
der Christian-Albrechts-Universität, Kiel
(Professor Dr. B. Behrens)

VERSUCHE ÜBER DIE WIRKUNG VON PHARMAKA DES VEGETATIVEN NERVENSYSTEMS AUF DIE EMPFINDLICHKEIT DER GONADEN FÜR CHORIONGONADOTROPINE

Von

J. Drescher und G. Zetler

Da die Funktion der Gonaden von den Gonadotropinen stimuliert wird, könnte sich ein etwaiger Einfluss des vegetativen Nervensystems sowohl auf die Produktion und Ausschüttung der Gonadotropine als auch die Empfindlichkeit der Gonaden für die Gonadotropine richten. Es ist nicht bekannt, welche dieser beider Möglichkeiten vorherrscht; dass die Funktion des HVL von Zentren im Zwischenhirn gelenkt wird, ist so gut wie sicher, ein direkter Einfluss des peripheren vegetativen Nervensystems hierauf dürfte jedoch wenig wahrscheinlich sein. Deshalb untersuchten wir, ob Reizung oder Lähmung des Sympathicus oder Parasympathicus die Empfindlichkeit der Gonaden für die Gonadotropine verändert. Eine grundlegende Bearbeitung dieses Problems existiert unseres Wissens nicht, nur wenige experimentelle Arbeiten berühren diese Frage; diese Arbeiten werden in der Diskussion besprochen.

MATERIAL UND METHODE

A) *Versuche an 72 männlichen Kröten (Bufo bufo L.).*

Die Kröten hatten ein Gewicht von 16–23 g und entstammten einem Freilandterrarium; sämtliche Untersuchungen fanden im Juli statt. Zwischen den Versuchen lagen jeweils 14 Tage. Am Versuchstag wurde ein Leerversuch (Gonadotropine ohne Pharmakon) und ein Hauptversuch (Gonadotropine mit vorheriger Gabe eines Pharmakons) durchgeführt. Zuvor hatten wir Tiere mit Spermatorrhoe durch Untersuchung des Kloakeninhalts eliminiert. Der Versuchsanordnung lag folgendes Schema zugrunde: