Kugeln 38 mm. Die selbstverfertigte Plexiglaskammer für das isolierte Organ weist einen Durchmesser von 8 cm, eine Höhe von 6 cm auf. Beim Schlauchmaterial handelt es sich um Spezial-Silikon-Schlauch mit den Durchmessern von 9 und 5 mm (Firma Haska, Bern). Die Nylonfilter entstammen Infusionsbestecken.

Summary. A device for perfusion of isolated organs is described, with which it is possible to perform short pulse labelling experiments or experiments on reversibility of various influences on one organ in one experiment. In principle there is a double circulation. Each circulation may be used *per se* to perfuse the organ, one after the other, without danger of mixing the perfusates.

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Medizinisch-chemisches Institut und Institut für klinische Eiweissforschung der Universität Bern (Schweiz), 7. August 1964.

[7] Wir danken dem Schweizerischen Nationalfonds zur Förderung der wissenschaftlichen Forschung für die Unterstützung dieser Arbeit.

A Bioassay for Insulin Using the *in situ* Mouse Diaphragm

Serum insulin-like activity (ILA) may be determined by a number of *in vitro* bioassays, the majority of these bioassays using either the rat diaphragm[1,2] or the epididymal fat pad[3,4]. There has not yet been a direct comparison of the *in vivo* and *in vitro* response to insulin of a tissue used in an *in vitro* bioassay.

A comparison is presented between the response to insulin of the *in situ* mouse diaphragm and the response to insulin of the *in vitro* mouse diaphragm. In both studies the diaphragm glycogen content is the insulin sensitive metameter. The response of the *in situ* mouse diaphragm is sufficiently sensitive and linear for this system to be used as an insulin assay.

The *in vitro* studies have been carried out using the *in vitro* mouse diaphragm assay of MOODY and FELBER[5]. The *in situ* assay is based on the demonstration by RAPHAELSON[6] that, in the rat, an intraperitoneal injection of insulin increases the diaphragm glycogen content.

Four characteristics of the systems are compared: the sensitivity, the linearity of the response to insulin, the kinetics of the response to insulin and the response to serum. The *in situ* system is more sensitive to insulin than the *in vitro* system. The kinetics and the response to serum of the two systems are very similar.

Materials and methods. Mice: Fasting male albino mice of between 13 and 20 g are used; at this weight the mice are immature and have little adipose tissue.

Buffer: Krebs-Ringer bicarbonate buffer pH 7.4[7] containing 2.0 mg gelatine and 3.0 mg glucose per ml is used.

Standard insulin solutions: Standard insulin solutions in the range 1 to 500 μU/ml are made up daily by dilution, in buffer, of a stock of 1 mg/ml beef insulin[8].

Experiment design: 0.5 ml of sample is injected into each mouse. For semi-quantitative experiments one mouse is used for each point; when higher precision is required at least two mice are injected with each sample.

Injection of the mice: Before injection the samples are gassed with 95% oxygen-5% carbon dioxide and warmed to 37°C. A Hamilton syringe, fitted with a No. 20 detachable needle, is used for the injection. The mice are gripped behind the ears and held stomach uppermost by an assistant. The injections are made into the peritoneal cavity through the abdominal wall. The incubation is timed from the withdrawal of the needle until the mouse is killed by decapitation.

Determination of the glycogen: The diaphragms are removed, in halves, within 1 min of the death of the mice. The glycogen of the hemidiaphragms is then prepared and determined as described elsewhere[5]. The values for the diaphragm glycogen loading are expressed as μg of glycogen per mg wet tissue.

In vitro technique: *In vitro* incubations are carried out as already described[5] using the formation of glycogen by isolated hemidiaphragms as the insulin sensitive metameter.

Results. The minimum detectable dose in the *in situ* assay is 1 μU/ml of beef insulin. The values for the glycogen content of *in situ* diaphragms after incubation for 30 min following injection of 0.5 ml of 1μU/ml insulin are significantly greater ($p = 0.001$) than the values obtained following injection with buffer alone (see Table I).

Table I. Response of the *in situ* mouse diaphragm to injection with Kreb's buffer and with 1 μU/ml of beef insulin

	Kreb's buffer	1 μU/ml of beef insulin
	1.16	1.48
	1.19	2.04
	0.45	2.07
	0.70	2.46
Mean values	0.88	2.01

The probability that the two responses are the same is less than 0.001. Each value represents the mean response, as μg of glycogen/mg, of one mouse. The incubations were carried out for 30 min.

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[2] P. J. RANDLE, Brit. med. J. *1954 i*, 1237.
[3] A. E. RENOLD, D. B. MARTIN, X. M. DAGENALS, J. STEINKE, R. J. NICKERSON, and M. C. SHEPS, J. clin. Invest. *39*, 1487 (1960).
[4] E. G. BALL and M. A. MERRIL, Endocrinol. *69*, 596 (1961).
[5] A. J. MOODY and J.-P. FELBER, Exper. *20*, 105 (1964).
[6] A. E. RENOLD and J. STEINKE, *Fortschritte der Diabetesforschung*. I. Symposium des Deutschen Diabetes Komitee (1963).
[7] W. W. UMBREIT, R. H. BURRIS, and J. F. STAUFFER, *Manometric Techniques and Tissue Metabolism* (Burgess Publishing Co., Minneapolis 1957), p. 149.
[8] Burroughs Welcome are thanked for their gift of beef insulin.

The glycogen loading of the *in situ* diaphragms shows a small rise, compared with an untreated mouse, when the mice are injected with buffer alone. The diaphragm glycogen content of a fasting mouse is between 0.5 and 1.0 μg/mg wet tissue. After injection with 0.5 ml of buffer alone, the level rises to between 0.7 and 1.5 μg of glycogen per mg wet tissues. The mouse pancreas seems to be relatively insensitive to presence of high peritoneal glucose concentrations.

Standard curves of the *in situ* assay are shown in Figure 1. The response, as μg of glycogen/mg wet tissue, is not linear when plotted against the log of the insulin dose. The curve consists of two sectors: a low slope sector from 1 μU/ml to 25 μU/ml and a sector of steeper slope from 25 μU/ml upwards. Replotting the data as μg of glycogen against a linear scale of insulin concentration straightens the curve (Figure 1). A similar assay departure from linearity exists in the *in vitro* assay.

Figure 2 shows the results obtained by using the same standards of insulin in both assays on the same day. Three serum samples were included in each assay. The glycogen levels are in the same range in both assay and the serum ILA values are not very different.

Kinetic studies have been carried out on the effect of peritoneal injections of insulin and of serum on the glycogen loading of the *in situ* diaphragm. Samples used in the studies were: buffer alone, 25μU and 100 μU/ml beef insulin and a serum sample. Incubation times of 5, 15, 30, 60 and 90 min were used. The results of these studies are shown in Figure 3. It can be seen that there is a response to 25 μU of insulin after 5 min and that the response to the two insulin samples and to the serum follow the same time course with a peak between 30 and 60 min. Replotting the data as a family of dose response curves shows that the steepness of the curve varies with time.

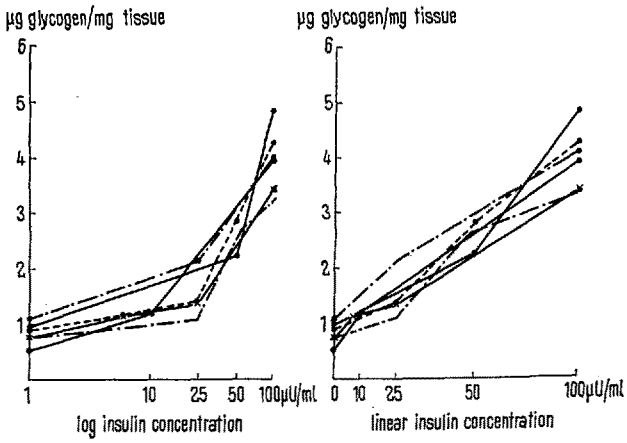

Fig. 1. Standard curves for the *in situ* assay. Each curve is plotted as glycogen loading against log insulin concentration (on the left) and as glycogen loading against a linear plot of insulin (on the right). Each point is the mean response of two mice after injection of 0.5 ml of sample per mouse. The period of incubation was 30 min.

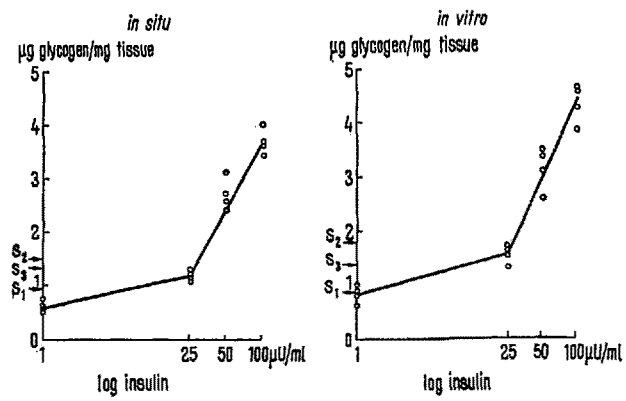

Fig. 2. A comparison of the standard curves obtained *in situ* and *in vitro* using the same standards of insulin on the same day. The period of incubation in both assays was 30 min. Each point represents one hemi-diaphragm. The values for the total ILA, in μU/ml, determined in the three serum samples are as follows:

Serum	ILA *in situ*	ILA *in vitro*
1	5	2
2	25	28
3	25	25

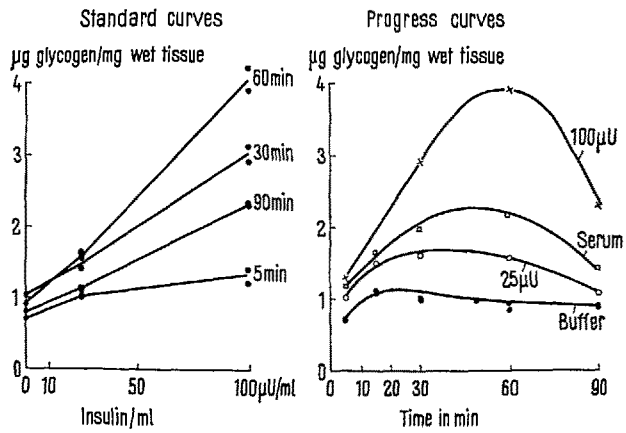

Fig. 3. Kinetic response of the *in situ* mouse diaphragm to insulin. The glycogen loading is plotted against the time of incubation for the dose levels of 0.25 and 100 μU/ml of insulin. The data are also plotted as a family of standard curves. Each point represents the mean value for the hemi-diaphragms from two mice.

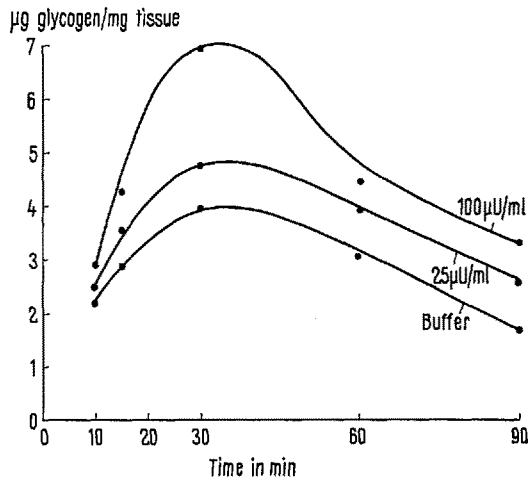

Fig. 4. Kinetic response of the *in vitro* mouse diaphragm to insulin. The glycogen loading of the diaphragms is plotted against time for the dose levels of 0, 25 and 100 μU/ml of insulin. Each point represents the mean value for four hemi-diaphragms.

A similar study has been carried out using *in vitro* incubations of 10, 20, 30, 60 and 90 min. Figure 4 shows that *in vitro* the time course of the response is similar to that of the *in situ* response; the difference being that the response curves are steeper *in situ* then *in vitro*.

Table II. Comparison of the total ILA (as μU/ml) determined in whole human serum by the *in situ* and *in vitro* diaphragm assays

Serum number	*in situ* ILA	*in vitro* ILA
1	5[a]	2[a]
	10	0
2	30[a]	28[a]
	20	20
3	25[a]	25[a]
	15	15
4	20	15
5	10	10
6	10	5
7	25	15
8	20	25
9	30	25
10	5	20

Pairs of results marked [a] were carried out on the same day. All other determinations were carried out on different days. The values for the ILA were allocated by comparing the mean value for the serum with a standard curve of glycogen loading against a linear plot of standard insulin concentration.

The total ILAs of a series of serum samples has been determined by these two assays. The results are presented in Table II.

Discussion and conclusions. The glycogen content of the *in situ* diaphragm of the mouse increases in response to intraperitoneal injections of either insulin or human serum. The response is proportional to the amount of insulin injected and is linear within certain limits of concentration. The system is extremely sensitive, 1 μU/ml of insuling being the minimum detectable concentration. The kinetics and steepness of the response are comparable with those of the MOODY and FELBER *in vitro* diaphragm assay. Serum total ILAs determined by either bioassay are similar.

The assay presented here, using the *in situ* mouse diaphragm as insulin sensitive tissue, provides a valuable proof of the validity of serum ILA determinations carried out with the isolated mouse diaphragm. The isolation of the mouse diaphragm does not, to any marked degree, alter the sensitivity of the tissue to insulin.

There seems to be little interference in the *in situ* assay from endogenous insulin; it is possible that the mouse pancreas is relatively insensitive to glucose administered by peritoneal injection.

Résumé. Description d'une méthode de détermination de l'insuline utilisant le diaphragme de souris *in situ*. Les résultats concordent avec un essai similaire *in vitro*.

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Influence des stimulations auditives sur le comportement sexuel du taureau

Les taureaux utilisés dans les centres d'insémination artificielle manifestent souvent, après un certain temps, un négativisme croissant à l'égard de la vache lors de la récolte du sperme. Diverses méthodes ont été préconisées pour remédier à cette situation[1]. Un des procédés les plus courants consiste à faire monter par le taureau, un congénère mâle au lieu de la vache. Dans une série d'essais préliminaires, nous avons tenté de faire accepter à nouveau la vache par des taureaux rétifs en faisant entendre à ceux-ci au moyen d'un magnétophone, des beuglements de vache enregistrés en étable. La stimulation auditive était continue pendant toute la durée de présence des animaux dans le local de récolte, jusqu'à l'éjaculation éventuelle[2].

Ces expériences s'étalèrent, selon l'animal considéré, sur une période de 7 à 9 jours et faisaient suite à une période de contrôle préalable de 15 à 35 jours, au cours de laquelle la récolte du sperme avait eu lieu dans les conditions ordinaires, c'est-à-dire sans intervention de la stimulation auditive. Quatre taureaux furent observés systématiquement au cours de ces deux périodes, la monte sur congénère mâle restant prévue en cas de refus de la vache après 15 min environ.

Les résultats des observations pratiquées au cours de la période de contrôle préalable, au sujet de l'acceptation de la vache par les taureaux, figurent au Tableau I.

Bien que les exigences de la récolte nous aient obligés, sauf dans un cas, à tenir compte des habitudes des animaux en les faisant monter assez fréquemment un congénère mâle, la proportion d'essais concluants lors de l'utilisation de la vache est très faible. Elle n'atteint que 28% pour les quatre animaux. Par contre, les essais avec taureau sont concluants à 100%.

Les résultats correspondants obtenus au cours des essais comportant la stimulation auditive, on été consignés au Tableau II.

On notera que le nombre total d'essais est moitié moindre qu'au cours de la période de contrôle préalable. Cette différence doit rester présente à l'esprit si l'on veut apprécier correctement les proportions de montes concluantes figurant au Tableau II. Toutefois, le nombre total d'essais avec vache n'est inférieur que de 4 unités au nombre total de ces mêmes essais au cours de la première période, ce qui rend les résultats comparables sous ce rapport.

Ceci dit, il est indéniable que les résultats obtenus pour les trois premiers taureaux sont nettement plus favorables.

[1] B. M. KERRUISH, Brit. J. anim. Behav. *3*, 125 (1955). – J. J. M. L. CROMBACH, Z. Tierzücht. Züchtungsbiol. *75*, 331 (1961).

[2] L'utilisation de grognements caractéristiques d'un verrat sexuellement motivé, émis par un magnétophone, entraîne immédiatement l'immobilisation de la truie en oestrus, en l'absence de stimuli visuels et olfactifs (J. P. SIGNORET, F. DU MESNIL DU BUISSON et R. G. BUSNEL, C.R. Acad. Sci. *250*, 1355 (1960).