vaccine showed myocarditis. Earlier studies[9] showed the occurrence of spontaneous myocarditis in this strain, although this was not evident in the present study and only one of the Lewis rats showed such lesions. In previous studies polymorphonuclear leucocytes were rarely found in the lesions; however, in this experiment they were present in large numbers in all the animals graded as +++. The presence of abundant polymorphonuclear leucocytes is probably related to the massive myocardial necrosis present and not to infection, as no microorganisms were found in the sections examined[10].

Zusammenfassung. Nachweis, dass Pertussis Impfstoff die Neigung zur Entstehung experimenteller Myokarditis bei Inzucht-Lewis-Ratten, die mit Kaninchen- und Rattenherzextrakten in vollständig Freund'schem Adjuvans immunisiert wurden, verstärkt. Keine zunehmende Neigung zur Entwicklung der Krankheit wurde hingegen festgestellt, wenn wahllos gezüchtete, genetisch nicht verwandte Ratten (Hebrew University strain «Sabra») in derselben Weise behandelt wurden.

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[9] A. Laufer, E. Rosenmann and A. M. Davies, Br. J. exp. Path. *47*, 605 (1966).

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Effect of Caerulein Infusion on Glucagon Secretion in the Dog[1]

Previous research[2] has shown that in the dog caerulein is the most potent stimulant of insulin secretion so far known: in fact, the threshold dose was 0.5–1 ng/kg per min by i.v. infusion and 5–10 ng/kg by single i.v. injection. On a molar basis, the insulin stimulant activity of caerulein was 2–3 times larger than that of cholecystokinin-pancreozymin.

Unger, Ketterer, Dupré and Eisentraut[3] demonstrated that i.v. infusion of cholecystokinin, but not of secretin or gastrin I, stimulated glucagon secretion in the dog. Completing our preceding data, we have studied in the present experiments the effect of caerulein infusion on glucagon secretion in the dog.

Materials and methods. Preparation of 6 mongrel dogs, insulin and glucose titration were carried out exactly as described in a previous paper[2]; glucagon concentration in the pancreatico-duodenal venous plasma was measured by the radio-immuno assay of Unger, Eisentraut, McCall and Madison[4].

Caerulein (prepared at the Farmitalia Laboratories for Basic Research, Milan) was dissolved in physiological saline solution and infused in the femoral vein at a rate of 2 ng/kg per min for periods of 30 min.

Results and discussion. The Table summarizes the results of our experiments. The tabulated data show that at the doses used caerulein increased glucagon concentration in the pancreatico-duodenal venous plasma. The increase was already evident 5 min after the infusion started and reached a peak after 20–30 min. When the infusion was discontinued, glucagon concentration returned to the basal level in 15–30 min.

Immuno-reactive insulin concentration in the pancreatico-duodenal venous blood increased during caerulein infusion, and the increments (about 2.5 times the base levels) were of the same magnitude as those observed in the preceding experiments[2]. Arterial glucose remained practically unmodified, confirming our previous results where only doses of caerulein higher than 5 ng/kg per min produced about a 100% increase in glycemia, with no evident relation to the dose. A typical experiment is shown in the Figure.

From present results it appears that caerulein strongly stimulates not only insulin but also glucagon secretion, thus once again confirming that the activity spectrum of caerulein exactly covers that of cholecystokinin.

Unfortunately, it was not possible to carry out comparative experiments with cholecystokinin. However, we can observe that Unger et al.[3] infusing cholecystokinin at a rate of 30 Ivy dog units/min (about 10 µg/min) obtained an increment in glucagon concentration in the pancreatico-duodenal venous plasma which was of the same order of magnitude as that we obtained in the present experiments with the infusion of 2 ng/kg per min (about 30 ng/min) of caerulein.

Arterial glucose remained practically unmodified after caerulein infusion. Similar results were obtained by Buchanan, Vance, Morgan and Williams[5] who injected in the dog 2.5–5 Ivy dog units/kg of cholecystokinin i.v. and observed a 2.3 and 3.2 times increase in glucagon and insulin concentration respectively, with a very small increase in arterial glucose (about 25%) which reached a peak 10 min after the maximum hormonal response.

This is not surprising if one keeps in mind that Unger et al.[3] obtained in the dog, after rapid endoportal injection, a 50% increase in glycemia only with doses of glucagon of 1 µg, which far exceeded the amount of glucagon (estimated at about 30 ng/ml) released in our experiments. Moreover, Meade, Kneubuhler, Schulte and Barboriak[6] did not find any change in arterial glucose following i.v. infusion of 1 Ivy dog unit/kg/min of cholecystokinin, in spite of the 30–40 fold substantial increase in portal insulin concentration elicited by the hormone.

It is hence evident that the amount of glucagon released by caerulein in the course of our experiments is too small to elicit an appreciable change in blood sugar levels. On

[1] This work was supported by grants from the Consiglio Nazionale delle Ricerche, Rome.

[2] G. Bertaccini, G. De Caro and P. Melchiorri, Br. J. Pharmac., in press (1970).

[3] R. H. Unger, H. Ketterer, J. Dupré and A. M. Eisentraut, J. clin. Invest. *46*, 630 (1967).

[4] R. H. Unger, A. M. Eisentraut, M. S. McCall and L. L. Madison, J. clin. Invest. *40*, 1280 (1961).

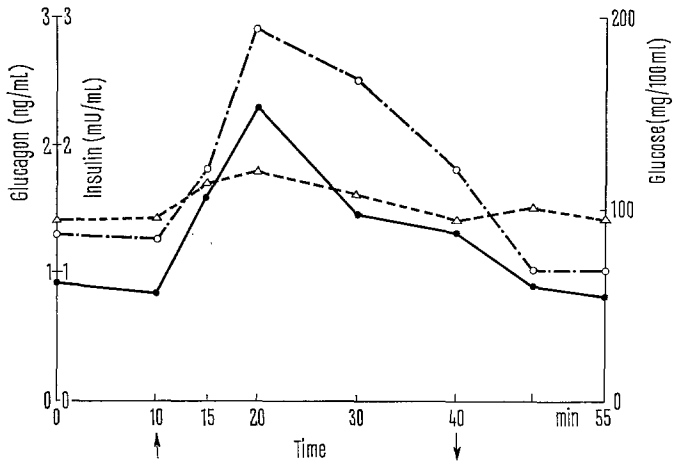
[5] K. D. Buchanan, J. E. Vance, A. Morgan and R. H. Williams, Am. J. Physiol. *215*, 1293 (1968).

[6] R. C. Meade, H. A. Kneubuhler, W. J. Schulte and J. J. Barboriak, Diabetes *16*, 141 (1967).

Effect of caerulein infusion on glucagon and insulin pancreatico-duodenal venous plasma concentration and on arterial glucose

	Glucagon (ng/ml)	Insulin (μU/ml plasma)	Glucose (mg/100 ml)
Physiological saline infusion	0.97 ± 0.62 $P < 0.05$ [a]	1450 ± 540 $P < 0.01$ [a]	95 ± 15 $P > 0.05$ [a]
Caerulein infusion (2 ng/kg/min)	3.30 ± 1.29	3700 ± 775	110 ± 20

[a] Student's t test. Figures are mean values at the maximum effect time.



Effects of infusion of caerulein at the rate of 2 ng/kg/min on glucagon (●—●) and insulin (○–·–○) concentration in the pancreaticoduodenal venous plasma and on arterial glucose (△– – –△). At the first arrow, infusion started, at the second arrow, infusion stopped.

the other hand, insulin and glucagon could well have counteracted reciprocally their effects on glycemia.

Present experiments do not contribute to the solution of the problem whether glucagon release is primary or secondary to the secretion of insulin. However, it may be observed that UNGER et al.[3] found that endoportal infusion of cholecystokinin (30 Ivy dog U/min/dog) produced a sharp increment in insulin (840%) as well as, although to a less extent, in glucagon (80%) and glucose concentration (60%); on the contrary, secretin infusion (10 U/min/dog) strongly enhanced (280%) insulin release but left unmodified pancreatico-duodenal venous glucagon and arterial glucose concentration. Also rapid endoportal injection of gastrin (60 μg/dog) increased insulin (400 to 1000%) but not glucagon and glucose concentration.

As stated above, in our present experiments caerulein infusion enhanced insulin (155%) as well as glucagon concentration (240%), with negligible changes in arterial glucose concentration (15% increase). This may signify that at least under our experimental conditions there is no relationship between the secretion of the two hormones. Thus, both cholecystokinin and much more caerulein are likely to possess a primary stimulant action both on the β and α cells of the pancreatic islets.

Riassunto. L'infusione di ceruleina (2 ng/kg/min) produce nel cane un significativo aumento della concentrazione del glucagone, oltre che della insulina, nella vena pancreatico-duodenale superiore, senza significative modificazioni della glicemia.

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Failure to Stimulate Significant Cortisol or Growth Hormone Secretion in Man by Ether Infusion

The tests for assessing pituitary ACTH reserve presently available for clinical use[1–3] are not completely satisfactory. The Metopirone test[1] endangers the patient with the possible crisis of hypocortisolism. Its principal failing is that it indicates the ability of the pituitary to secrete increased amounts of ACTH in response to a depression of blood cortisol levels but does not reflect the ability to increase ACTH secretion in response to stress. These 2 stimuli (decreased blood cortisol and stress) probably activate ACTH secretion by quite different mechanisms[4]. The Metopirone test has indicated a markedly depressed ACTH reserve in some patients who have subsequently responded to surgical stress with a normal increase in adrenal secretion[1]. Pyrogen[5] tests the ability of stress to increase ACTH secretion, but causes fever and other unpleasant side effects. Both tests are time-consuming and require hospitalization of the patient. Insulin-induced hypoglycemia[6] is potentially dangerous and is accompanied by disagreeable symptoms.

In a search for a more suitable diagnostic tool, we studied the effect of i.v. administered ether on the plasma 17-OHCS (17-hydroxy corticosteroids)[7]. Ether