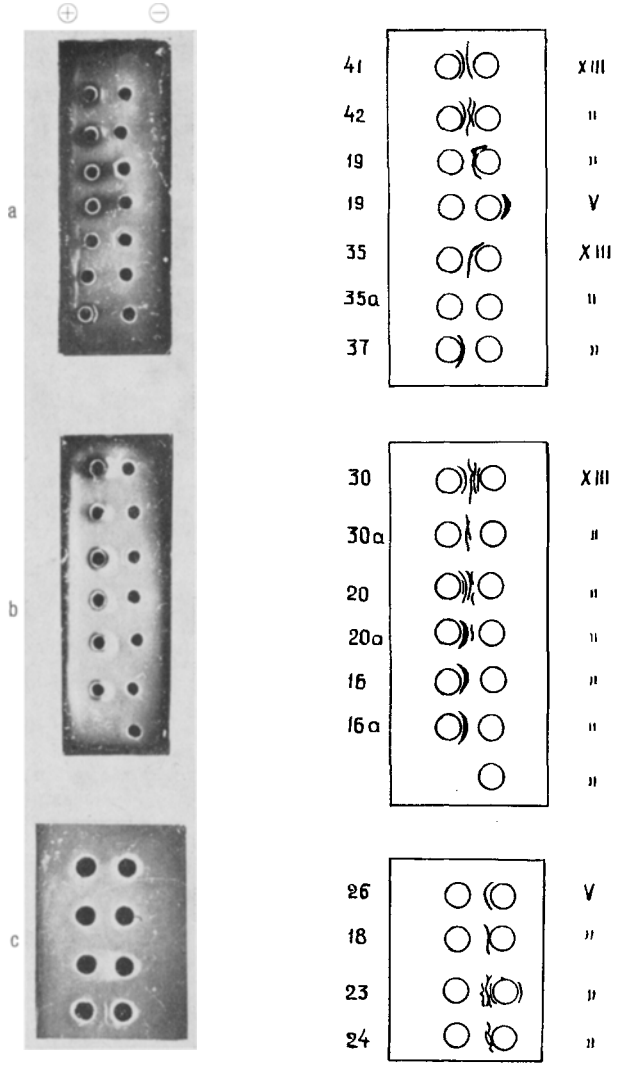

Fig. 4. Immuno-osmophoresis test. Antigens: purified FPV concentrated 13 times (XIII) and normal chorioallantoic membrane (V). Sera: FPV (30), FPV V (20) and S (16), NDV (23, 24), Sendai (35, 37, 41, 42), normal chorion allantoic membrane (19), human group B (26); index 'a'-exhausted with normal chorioallantoic membrane antigens.

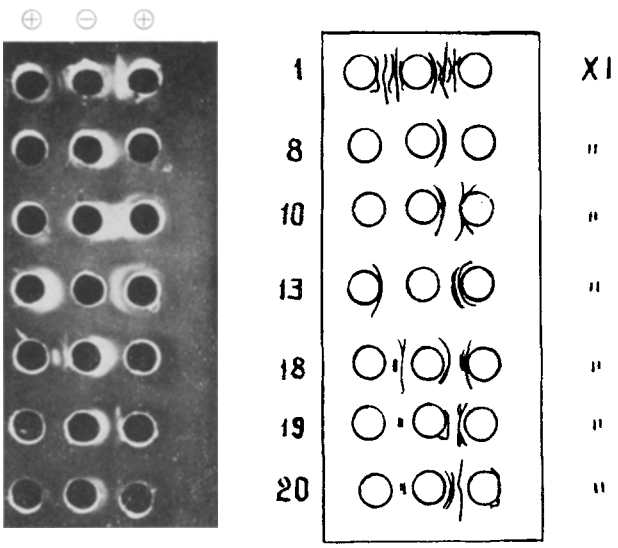

Fig. 5. Biphasic immuno-osmophoresis test. Antigen: purified FPV concentrated 11 times (XI). Sera: FPV (1, 10, 13) and FPV V sera (20), normal charioallantoic (18, 19) and standard hemolytic sera.

Выводы. Для выявления клеточных антигенов в составе вирусов классической чумы птиц, болезни Ньюкасла и Сендай были применены методы диффузии в агаре, иммуноэлектрофореза и иммуноосмофореза. С помощью этих методов удалось выявить в составе вирионов несколько клеточных антигенов, включая видоспецифический, группоспецифический А и антиген Форсмана.

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Experimental Myocarditis: Enhancement by the Use of Pertussis Vaccine in Lewis Rats[1]

It has been shown that Lewis rats are more susceptible than other strains to a number of auto-immune disease[2]. In addition, pertussis vaccine is known to accelerate and intensify certain experimental auto-immune diseases when given in addition to the antigen adjuvant emulsion[3–6].

In previous studies, 28% of Hebrew University stock rats immunized with heart extracts in complete Freund's adjuvant, developed myocarditis[7]. The present experiment was designed in order to establish whether Lewis rats were more susceptible to the development of myocarditis than the Hebrew University stock rats and whether pertussis vaccine has any augmenting effect on this process.

Materials and methods. Animals: Inbred Lewis rats (Microbiological Associates, Bethesda, Md.) and 'Sabra' rats of the Hebrew University outbred stock, of both sexes and weighing 150–200 g, were used.

Antigens: Rabbit and rat hearts were stored at — 20 °C, thawed before use and a 33% homogenate in saline prepared in a Sorvall omnimixer at 4 °C. The homogenate was filtered through 2 layers of gauze and the protein content determined. The antigens were mixed in equal volumes with Freund's complete adjuvant (Difco) enriched with 4 mg/ml of *M. tuberculosis* human type C, DT, PN (kindly supplied by the Ministry of Agriculture, Fisheries and Food Control Veterinary Lab., Weybridge, Surrey, England). The final protein content of the adjuvant emulsion was 20–25 mg protein/ml.

Pertussis vaccine was kindly supplied by RAFA Ltd. Laboratories, Jerusalem, and contained 228×10^9 *Bordetella* per ml.

Myocarditis in rats sensitized to rabbit and rat heart-adjuvant emulsion with or without pertussis vaccine

Rat strain	Heart antigen	Adjuvant	Pertussis vaccine	Degree of myocarditis[a] 0	+	++	+++	Rats with myocarditis/ total injected
Lewis	Rabbit	+	+	4	5	10	4	19/23
	Rabbit	+	−	6	1	0	0	1/7
	Rat	+	+	5	6	2	0	8/13
	None	+	+	22	1	0	0	1/23
	None	−	+	8	0	0	0	0/8
Hebrew	Rabbit	+	+	8	1	1	0	2/10
University	Rabbit	+	−	5	0	1	0	1/6
'Sabra'	None	+	+	5	0	0	0	0/5
	None	+	−	5	0	0	0	0/5

[a] After 14 days. For grading of lesions see text.

Immunization: Rats were injected with 0.1 ml of the antigen adjuvant emulsion into the foot pad of each hind leg (a total of 4–5 mg protein per rat), followed by an injection of 0.05 ml pertussis vaccine into the dorsum of each hind leg. Control rats were injected with the same amount of saline adjuvant emulsion with or without pertussis.

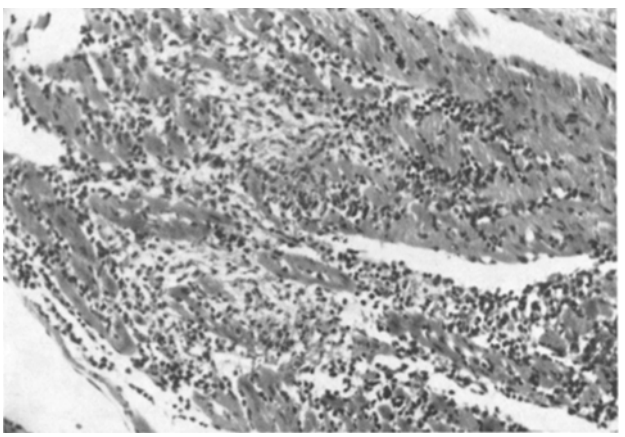
Histological examination: 14 days after immunization the animals were killed by ether, the hearts were removed and fixed in 4% neutral formalin, sectioned, and stained with haematoxylin and eosin.

Tanned cell haemagglutination was performed as described elsewhere[8].

Results. The results are summarized in the Table. Only 2 of 13 rats developed myocarditis without the addition of pertussis vaccine. The addition of pertussis vaccine had a marked enhancing effect upon the development of the disease in the Lewis rats but not in the Hebrew University strain. In the latter, only 2 of the 10 animals immunized with rabbit heart and pertussis vaccine developed myocarditis. Lewis rats immunized with xenogeneic (rabbit) heart developed myocarditis more frequently and to a greater extent than those immunized with allogeneic (rat) heart.

The histological changes in the myocardium were fundamentally similar but varied in their intensity and distribution. The most marked changes consisted of both focal and diffuse interstitial cellular infiltration, classified as +++ (Figure). The lesions were irregularly distributed in the myocardium and consisted of lymphoid and histiocytic cells accompanied by muscle necrosis. In some of these focal lesions polymorphonuclear leucocytes were also present. In other sections, there were fewer focal lesions and no diffuse lesions. Minimal muscle necrosis was evident and in general the inflammatory infiltrate was less striking. These lesions were classified as +. No such lesions were found in other organs examined. Tanned haemagglutination tests showed some positive sera but no correlation with the myocardial lesion. This finding corresponds with a previous study[7].

Discussion. In the present study we have demonstrated that pertussis vaccine potentiates the development of myocarditis in Lewis rats. These results accord with those of others in experimental allergic encephalomyelitis[4], adrenalitis[5], thyroiditis[3] and aspermatogenesis[6]. This study also shows the difference in susceptibility to myocarditis of the inbred Lewis rats as opposed to the outbred Hebrew University strain. Rabbit heart extracts proved to be a more potent antigen than rat heart. This finding confirms those of earlier studies[7], although it differs from the results obtained by other workers using thyroid and adrenal antigens[3,5]. Only 2 of the 10 rats of the Hebrew University strain treated with pertussis



Diffuse cellular infiltration of myocardium, graded +++. H. & E., × 150.

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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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[10] Acknowledgement. We thank Igal Gery, from the Department of Medical Ecology, for valuable assistance.

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

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