clear plaques and a CPE which rapidly destroys all of the cells(1).

It was not apparent from these observations, however, why plaques formed by SV2 and SV6 were smaller and slower in appearing than those of SV42 and SV49. The studies presented here show that all 4 viruses adsorb to, penetrate and multiply in PMK cells at approximately the same rate. The one difference noted was that SV2 and SV6 tended to remain intracellular while SV42 and SV49 were readily released into the medium. Therefore spread of infection from cell to cell in a plaquing system would be facilitated for the latter 2 viruses and it might be expected they would produce larger plaques.

A second difference noted was that SV6, SV42 and SV49 produced a considerably higher yield of virus per cell than did SV2.

Finally, it was found that SV6 was inhibited by the agar inhibitor in the overlay medium. Even with protamine in the medium, however, plaque size did not approach that of SV42 and SV49.

One further characteristic of these viruses might be noted. SV2 and SV42 are more heat labile than SV6 and SV49(1,6). This may explain why SV49 consistently produces larger plaques than SV42 even though the virus yield is higher for SV42.

In conclusion, it can be said that while gross morphology of the plaques formed by SV2, SV6, SV42 and SV49 is related to the type of CPE observed in cell cultures under liquid media, the plaque size is determined by a number of factors including virus yield per cell, ability of the virus to be released from the cell once synthesized, influence of factors such as protamine in the overlay medium and possibly thermal stability of the virus.

Summary. A study was made of the rate of adsorption, penetration and multiplication in PMK cell cultures by 4 simian enteroviruses which form different kinds of plaques. The effect of protamine on plaque formation by these viruses was also noted. The results obtained were used to explain differences observed in the size of plaques formed.

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Effect of Graded Levels of Insulin on Feed Consumption in Normal Female Rats.* (30667)

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The variation in the voluntary feed consumption of normal mature female rats of Sprague-Dawley-Rolfsmeyer strain has been observed(1). The mean feed intake of 265 g rats was 5.3 g/100 g body weight (bw) with a range from 2.9 to 7.5 g/100 g bw/day. While the neural regulation of feed intake has been studied extensively(2) the possible role of the endocrine glands and their hormones has been neglected. It is interesting to speculate as to the cause of the difference in feed consumption of individual animals. It has been suggested that the difference may be due, in part, to variation in the secretion rate of various hormones.

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The effect of the exogenous administration of various hormones upon feed consumption is of interest, also, in interpreting the role of the hormones upon mammary gland growth and the increase in milk secretion. If a given hormone stimulates an increase in feed consumption, part of the effect induced may be ascribed to the greater intake of nutrients upon the process studied. It has been shown that 3 units of insulin/day increased mammary gland DNA by 32%(3) and the intensity of milk secretion, a mean of 42% between days 14 and 20 of lactation(4). Quantitative data is lacking as to the effect of insulin upon feed consumption in the rat. It is of interest to determine to what extent these observations may be due to feed intake. It has been suggested that metabolic hypoglycemia may lead to hyperphagia(5).

In our initial study the effect of several hormones on the feed consumption of rats was studied. L-thyroxine (L-T_4) at a level of 3.0 μg/100 g body weight (bw)/day failed to stimulate a significant increase in feed consumption during a period of 9 days (6). However, in a previous long time trial, feed consumption in female rats at this level of L-T_4 increased by 11.1%(7).

Hydrocortisone acetate (HCA) at a level of 500 μg/day during a period of 9 days stimulated a significant increase of 6.3% in feed consumption. Bovine growth hormone (GH) at a level of 1 mg/day for 16 days stimulated a body weight gain of 24 g. While feed consumption increased, there was a decrease of 4.0%/100 g bw. The combination of 2 or 3 hormones in all cases depressed food consumption/100 g bw(6).

It has been shown that the secretion rate of several hormones is increased during lactation, including thyroxine(8), adrenal glucocorticoids(9), lactogen(10), growth hormone(11) and possibly insulin(4). If one determined the influence of these hormones separately and together on feed intake one might estimate the maintenance requirement of the lactating animals.

The present paper presents data upon the effect of graded levels of insulin on feed consumption in normal adult female rats.

Materials and methods. Fifty-four nulliparous mature female rats of the Sprague-Dawley-Rolfsmeyer strain weighing a mean of 241 g were placed separately in metabolism cages constructed so as to prevent coprophagy and feed wastage. Temperature was maintained at 78 $\pm$ 1°F and the rats were artificially illuminated during daylight hours. Purina Lab. Chow with an energy value of 4.4 calories per gram and 23.4% total protein was fed during a preliminary period of 6 days and during the experimental periods to determine the mean daily feed consumption of each animal in the group. Protamine zinc insulin suspension (USP) (Lilly‡) was diluted in an alkaline saline solution. The animals were injected subcutaneously, once daily, at approximately the same time as follows: 1) 24 controls were injected with an alkaline saline solution, 2) 30 rats were injected with graded levels of insulin from 0.5 unit up to 6 units for periods of 6 days. The rats and left-over feed were weighed daily. The mean feed consumption was calculated per 100 g body weight.

Results. The 24 control rats weighing initially a mean of 240 g were maintained for a period of 78 days covering the experimental period. The mean daily feed consumption by 6-day periods is presented (Table I). It will be noted that the daily feed intake increased up to 14 to 16% over the preliminary period but closed at 11% above. This increase was related to the increase in body weight since the feed consumption/100 g bw remained essentially unaltered during the final 18 days. The control animals showed a mean body weight increase of 30 g during the experimental period. All animals survived. In the group of 30 experimental animals, normal feed consumption was determined during a period of 6 days prior to insulin administration. The mean body weight and daily feed consumption were respectively, 242 g and 5.7 g/100 g bw. With increasing levels of insulin, there was a gradual increase in the mean total daily feed intake up to 20.3 g in the surviving rats, an increase of 48%. During

‡ Kindly supplied by Eli Lilly and Co., Indianapolis, Ind.

TABLE I. Effect of Graded Levels of Insulin on Feed Consumption in Normal Female Rats.

Control							Experimental						
		Mean for 6 days (g)						Mean for 6 days (g)					
No. of surviving rats	Treatment	B.W.	F.C. per day	% of Inc. per day	F.C. per 100 g B.W.	% of Inc. per 100 g B.W.	No. of surviving rats	Treatment	B.W.	F.C. per day	% of Inc. per day	F.C. per 100 g B.W.	% of Inc. per 100 g B.W.
24	Normal	240	13.3	100	5.6	100	30	Normal	242	13.7	100	5.7	100
24	.3 Normal alkaline saline	242	14.4	8	6.0	7	30	.5 unit insulin	242	13.6	−1	5.6	−1
							30	1.0 "	247	14.1	3	5.7	1
24	"	244	13.4	0	5.5	−2	30	1.5 "	249	14.1	3	5.7	1
24	"	249	13.8	4	5.5	−1	30	2.0 "	254	14.9	9	5.9	4
24	"	250	14.2	6	5.7	1	30	2.5 "	260	15.7	15	6.0	7
24	"	261	15.2	14	5.8	4	30	3.0 "	262	15.8	16	6.0	7
24	"	260	14.8	11	5.7	2	29	3.5 "	267	16.4	20	6.2	9
24	"	264	15.3	14	5.8	4	28	4.0 "	274	17.6	29	6.4	14
24	"	264	15.1	13	5.7	3	27	4.5 "	282	18.3	34	6.5	15
24	"	263	15.5	16	5.9	6	24	5.0 "	289	18.2	33	6.3	11
24	"	266	14.9	12	5.6	1	19	5.5 "	292	19.1	40	6.6	16
24	"	268	14.4	8	5.4	−3	10	6.0 "	295	20.2	48	6.9	21
24	"	270	14.8	11	5.5	−1	10	No insulin	294	9.5	−31	3.2	−43

B.W. = body weight. F.C. = feed consumption.

the experimental period, the rats showed a mean gain of 53 g in body weight, an increase of 23 g over the control group. In spite of the greater increase in body weight, the insulin injected group showed a gradual increase in feed consumption/100 g bw. The percentage increase was 7% at the 3 unit level, 14% at the 4 unit level, 11% at 5 units, and 21% at 6 units.

In the experimental group, one rat died during the injection of 3.5 units. As the insulin was increased, a second rat died on 4 units, 1 on 4.5 units, 3 on 5 units, 5 on 5.5 units and 9 on 6.0 units. Insulin was withdrawn in the 10 surviving rats. Food consumption/day decreased 31% and 43%/100 g bw but the body weight remained the same. In order to determine if there was a relation between feed consumption and insulin tolerance, the mean feed consumption was determined in the groups of increasing tolerance. It will be noted that the groups showing increasing tolerance consumed 12 and 6% more feed during the control period (Table II). Analysis of variance shows that the effect of graded levels of insulin on feed consumption and body weight increase was significant at the 0.01 level.

Discussion. The mean normal feed consumption of a group of 24 female rats was determined at 6-day intervals during a period of 78 days. The initial mean body weight was 240 g and the final body weight was 270 g, a gain of 30 g. During this period, the mean daily feed intake increased slightly but on a 100 g bw basis, the feed intake was essentially constant indicating that the slight increase in feed consumption was related to the increase in body weight.

In the experimental group of 30 animals weighing a mean of 242 g, the gradual increase in insulin injected varying from 0.5 to 6.0 units for successive 6-day periods stimulated an increase in body weight to 295 g, an increase of 53 g and a difference of 23 g in comparison with the control group. Mean total feed consumption gradually increased from 13.7 g/day to 20.2 g/day on the 6 unit level. This is an increase of 48% in feed intake/day. That insulin stimulated feed intake in excess of increased growth rate was

TABLE II. Relation Between Feed Consumption and Insulin Tolerance.

Insulin tolerance	Initial (g) No. of animals	Mean body wt	Mean feed consumption	g/100 g body wt	% increase
Died between 3.5 to 5 units insulin	10	241	12.8	5.3	
Died between 5.5 to 6 units insulin	10	242	14.4	6.0	12
Survivors	10	245	13.9	5.7	6

shown by the feed intake of 5.7 g/100 g bw at the initial normal period to 6.9 g/100 g bw on the 6 unit level, an increase of 21%. All rats in the experimental group survived increasing levels of insulin up to 3 units. As the level was increased from 3.5 to 6.0 units, there was a gradual increase in the mortality rate indicating that an increasing number of rats were unable to withstand the hypoglycemic effects of insulin. To determine whether the increased insulin tolerance was related to their normal feed consumption, the groups were separated on the basis of insulin tolerance. It will be seen that the rats surviving on 6 units consumed only 6% more feed under normal conditions, whereas the intermediate group consumed 12% more feed (Table II). It would appear that high normal feed intake is not an important criteria of insulin tolerance.

It was shown in a previous study(4) that the daily injection of 3 units of insulin in lactating rats from day 7 to 19 stimulated increased milk yield of 38% on day 14, 25% on day 16, 26% on day 18 and 79% on day 20. That the increased yield of milk may be due in part to increased feed consumption is shown by the present study. At the 3 unit level of insulin, the rats ate 15.8% more feed/day and 7% more/100 g bw.

Summary. In a group of 24 normal mature female rats weighing 240 g, the mean daily food consumed was 13.3 g and 5.6 g/100 g body weight (bw). During a period of 78 days body weight increased 30 g. Feed consumption/day increased slightly during this period but it was related to increasing bw as shown by a lack of an increase/100 g bw. Mean daily feed intake was determined also in a group of 30 female rats weighing a mean of 242 g. Feed intake was 13.7 g/day and 5.7 g/100 g bw. During 78 days they gained 53 g. As the insulin level was increased daily feed consumption increased up to 20.2 g/day or a 48% increase and 6.9 g/100 g bw, an increase of 21%. This indicates that insulin increased feed intake in excess of the feed required for the gain in weight. All rats survived insulin at 3 unit level. As the insulin was increased further, an increasing number of rats failed to survive and at the 6 unit level only 10 rats survived.

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