The Influence of Energy Intake on Ribonucleic Acid Metabolism

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In a preceding paper (Munro & Naismith, 1953), we described the influence of energy intake on the protein metabolism of the rat. The caloric value of the diet was varied from about 850 to about 1700 kg.cal./sq.m. of body surface area and it was observed that, when the diet provided adequate amounts of protein, this change in energy intake was accompanied by a considerable alteration in nitrogen balance and in the amount of protein in the liver, high energy intakes being associated with positive nitrogen balances and larger amounts of liver protein. On the other hand, when the diet contained no protein, increments in energy intake had little or no influence on nitrogen balance, and, if anything, caused a reduction in the amount of protein in the liver. It was thought that the conditions provided in these experiments might be suitable for studying the nature of the alleged relationship between ribonucleic acid (RNA) and protein synthesis (Brachet, 1950; Caspersson, 1950). We have therefore investigated the influence of energy intake on the total amount of RNA in the liver and on the rate of incorporation of ^{32}P into liver RNA, both when the diet contained adequate amounts of protein and when it was free from protein. A preliminary account of these experiments has already appeared (Munro, Naismith & Wikramanayake, 1952).

METHODS

Total ribonucleic acid in the liver

The livers studied came from the rats used in the investigation of the relationship between energy intake and protein metabolism, described previously (Munro & Naismith, 1953). Full details of their selection and feeding and methods of analysis employed are given there. Briefly, male rats of about 250 g. body weight were given either a diet containing adequate amounts of protein or a protein-free diet, fed for the first week at a level of 1200 kg.cal./sq.m. of body surface area. Then for 4 days different rats received different energy intakes, produced by varying the amount of carbohydrate or fat in the diet. At the end of this period the rats were killed by exsanguination and the livers removed. The livers were then submitted to a modified Schmidt-Thannhauser separation procedure to give the amounts of protein, RNA, deoxyribonucleic acid and phospholipin present.

The first four experiments deal with the addition of carbohydrate or fat to protein-containing and to protein-free diets. These were carried out as separate experiments.

In a fifth (confirmatory) experiment, the effect of additions of carbohydrate to protein-containing and protein-free diets were studied simultaneously. In addition, a further confirmatory experiment was performed in which the effect of adding fat to a protein-free diet was compared with the effect of adding carbohydrate. This last experiment is not described in the paper on protein metabolism.

The amounts of ribonucleic acid found are expressed as RNA/liver. Since the rats were initially of the same size, consistent differences in RNA/liver can be ascribed to the diets used. Statistical analyses were carried out as before. The regression coefficients presented represent the change produced by an alteration in energy intake of 1000 kg.cal./sq.m. of body surface area. There were no significant deviations from linearity of regression.

Incorporation of ^{32}P into ribonucleic acid

Experiments were carried out on a different series of animals from those described above. Male albino rats were used, but the choice was not restricted to those of 250 g. body weight. Within each experiment, animals of closely similar weight were selected, but the mean initial weight varied in different experiments from about 180 to 230 g.

Except for two experiments (Table 6 and Fig. 3), the feeding arrangements were similar to those used in studying the influence of energy intake on total RNAP/liver. The energy intake was adjusted to 1200 kg.cal./sq.m. of body surface area for 1 week, and was then varied so that some rats received a low intake of energy whilst others received a high intake, the other dietary constituents being kept constant. Unless otherwise stated, these changes in energy intake were produced by varying the amount of carbohydrate in the diet. The rats were maintained at these different energy levels for 4 days. On the last day they were injected intramuscularly with ^{32}P as inorganic phosphate (10 μC /100 g. of body weight) and were killed by exsanguination under ether anaesthesia after 4, 8 or 24 hr., in most experiments at 24 hr. The liver was quickly perfused with 0.9% saline and then subjected to the slightly modified Schmidt-Thannhauser procedure described by Davidson & Smellie (1952). In this way an alkaline digest was obtained containing, amongst other things, deoxyribonucleic acid and ribonucleotides. The deoxyribonucleic acid was precipitated by bringing the digest to pH 3 with 60% (w/v) perchloric acid, and removed by centrifugation. The precipitate was washed twice with *N*-perchloric acid and the supernatant and washings combined for ionophoretic separation of the ribonucleotides. This was carried out by the procedure described by Davidson & Smellie (1952). In most cases, separation was only carried out over a 7 hr. period with a potential gradient of 15 V./cm. of filter paper. This is sufficient to provide a considerable separation of contaminant phosphorus compounds from the ribonucleotides, but

does not allow elution of the individual nucleotides separately. In some cases, we also submitted the digest to ionophoretic separation over a period of 18 hr. at a potential gradient of 11 V./cm. and obtained separation of individual nucleotides. After elution, P estimations and determinations of radioactivity were performed as described by Davidson, Frazer & Hutchison (1951). The radioactivity of the inorganic phosphate of the liver was obtained by precipitation of $\text{Mg}(\text{NH}_4)\text{PO}_4$, as described by Davidson *et al.* (1951); in view of the criticism of some forms of the $\text{Mg}(\text{NH}_4)\text{PO}_4$ precipitation procedure by Ennor & Rosenberg (1952), it should be noted that no carrier phosphate was used. In a few instances the specific activity of the acid-soluble organic phosphorus fraction (S_1 fraction of Davidson *et al.* 1951) was determined.

Fig. 1. Changes in liver ribonucleic acid produced by variations in intake of carbohydrate (●—●) or fat (○---○). The upper two curves represent the regression lines obtained when the diet contained protein. The lower two regression lines were obtained when the diet was free from protein. Each point is the mean result obtained with four or five rats.

The results have been expressed as specific activities (counts/min./100 $\mu\text{g. P}$) and also as relative specific activities (specific activity of organic phosphate P divided by the specific activity of inorganic P of liver multiplied by 100). The data have been analysed by recognized statistical procedures (Snedecor, 1946).

RESULTS

Energy intake and the quantity of ribonucleic acid in the liver

When the diet contained adequate amounts of protein, the amount of RNA in the liver varied considerably with energy intake (Fig. 1). This occurred whether carbohydrate or fat was the variable energy source in the diet, the regression coefficient being $+1.96 \text{ mg. RNAP/1000 kg.cal.}$ in the case of carbohydrate and $+2.01 \text{ mg. RNAP/1000 kg.cal.}$ in the case of fat; both coefficients were highly significant ($P < 0.01$). In the case of the protein-free diet, additions of carbohydrate caused a smaller, though still significant, rise in the amount of RNAP/liver ($+0.60 \text{ mg./1000 kg.cal.}$; $P < 0.01$) but additions of fat failed to do so ($-0.07 \text{ mg. RNAP/1000 kg.cal.}$; $P > 0.05$). The regression coefficients obtained in the case of the protein-free diets are both significantly less than those obtained with the protein-containing diets ($P < 0.01$). Comparison of the addition of carbohydrate to the protein-free diet with the addition of fat also suggests a difference in action, though here the level of statistical significance ($P = 0.05 - 0.02$) is not conclusive. These conclusions are based on the first four experiments which were not carried out at the same time. Confirmatory experiments were therefore performed in which different dietary groups were studied simultaneously. The first of these (Table 1) demonstrates that addition of carbohydrate to a protein-containing diet

Table 1. *The effect of changes in energy intake (carbohydrate) at different levels of protein intake on the ribonucleic acid content of the liver*

(Groups of ten rats.)		
Diet	Daily energy intake (kg.cal./sq.m.)	Total RNAP/liver (mg.)
Protein-containing	820	6.42
	1570	7.51
Protein-free	880	5.26
	1650	5.48

Analysis of variance indicates a highly significant ($P < 0.01$) effect of protein intake and of energy intake on the quantities of RNAP and a very significant difference in the influence of energy intake at the two levels of protein intake ($P < 0.01$ for interaction).

had a significantly greater influence on the RNA content of the liver than its addition to a protein-free diet. In a second experiment (Table 2) we compared the effect of adding carbohydrate to a protein-free diet with the effect of adding fat, and were unable to demonstrate a significant difference in their influence on the amount of RNA/liver. Thus the essential point established is that the amount of

RNA in the liver is much more sensitive to changes in energy intake when the diet contains protein than when it is devoid of protein.

Table 2. *Comparison of the effect on liver ribonucleic acid of adding carbohydrate or fat to a protein-free diet*
(Groups of five rats.)

Source of energy variation	Daily energy intake (kg.cal./sq.m.)	Total Liver RNAP/rat (mg.)
Carbohydrate	880	5.34
	1650	5.29
Fat	880	5.28
	1660	5.49

Analysis of variance does not reveal any significant difference between the action of carbohydrate and of fat.

Energy intake and ^{32}P incorporation into ribonucleic acid

The influence of increasing energy intakes on the incorporation of ^{32}P into the RNA of liver is illustrated in Table 3. The animals were killed 24 hr. after injection of the isotope. The phosphorus of RNA is eventually derived from liver inorganic phosphate, and the specific activity of RNAP will therefore be influenced by the specific activity of the inorganic phosphate; variations in this factor can be allowed for by expressing the results relative to the specific activity of liver inorganic phosphate, i.e. relative specific activity. In the case of rats receiving protein in the diet, the relative specific activity tends to fall as energy intake rises, whereas on the protein-free diet there is an appreciable rise in relative specific activity. An alternative method of correcting the specific activity of RNAP for differences in the activity of inorganic phosphate is the statistical procedure of covariance analysis (Snedecor, 1946); this allows one to adjust the observed specific activities of RNAP to give the values which would have been obtained if the specific activity of liver inorganic phosphate had been the same for all animals. The correction is made solely from evidence of the relationship between the specific activities of RNAP and inorganic phosphate provided by the experiment itself. In the present case, such an analysis (Table 3, column 7) confirms completely the picture obtained from the study of relative specific activity.

The experiment just described is representative of a series of observations on rats killed 24 hr. after injection of ^{32}P . Over the whole series, thirty-three animals received the protein-free diet and eighteen were given the protein-containing diet, energy intake ranging from 600 to 1900 kg.cal./sq.m. of body surface area. The change in relative specific activity induced by alterations in energy intake over this range is expressed by the regression coefficients/1000 kg.cal./sq.m., namely +10.3 for the

Table 3. *The effect of changes in energy intake (carbohydrate) on the uptake of radioactive phosphorus by liver ribonucleic acid 24 hr. after injection of ^{32}P*
(Groups of four rats.)

Diet	Mean initial body weight (g.)	Daily energy intake (kg.cal./sq.m.)	Specific activity of liver		Relative specific activity of RNAP	Specific activity of RNAP adjusted by covariance analysis (counts/min./100 $\mu\text{g. P}$)	Total radioactivity of liver RNAP, adjusted by covariance analysis (counts/min.)
			Specific activity of RNAP (counts/min./100 $\mu\text{g. P}$)	inorganic phosphate (counts/min./100 $\mu\text{g. P}$)			
Protein-containing	185	810	580	1527	37.9	683	22300
	186	1715	685	1896	35.8	646	27600
Protein-free	193	820	761	2004	38.2	680	21300
	189	1790	839	1747	48.4	857	31600

Analysis of variance shows that the effect of changes in energy intake was significantly different ($P < 0.05$) for interaction at the two levels of protein intake for the relative specific activity of RNAP and for the specific activity adjusted by covariance analysis. The total radioactivity/liver showed a highly significant response to energy intake ($P < 0.01$), but this effect was not significantly greater for the protein-free diet ($P > 0.05$ for interaction).

protein-free diet and +1.2 for the protein-containing diet. If we take the relative specific activity at 1200 kg.cal. as our reference standard, these regression coefficients represent a change of +26.5 and +3.4% respectively, in relative specific activity. The change in rate of incorporation is significant for the protein-free diet ($P < 0.01$) but not for the protein-containing diet.

Fig. 2. The effect of adding carbohydrate to a protein-free diet on the relative specific activity of ribonucleic acid phosphorus. The rats were injected with ³²P as orthophosphate 24 hr. before killing. Each point is the mean of three observations.

In order to confirm the magnitude of the change found in the case of the protein-free diet, a further experiment was carried out in which carbohydrate was added to a protein-free diet to give four levels of energy intake. The results obtained (Fig. 2) leave no doubt of the linear relationship between energy intake and relative specific activity of RNAP. From the calculation of the regression coefficient, it was ascertained that an increment of 1000 kg.cal./sq.m. of surface area resulted in a 27.8% alteration in relative specific activity, again using the value at 1200 kg.cal./sq.m. as our reference standard ($P < 0.05$).

The observations so far described concern only the values obtained 24 hr. after injection of labelled phosphorus. The same picture has, however, been observed with animals killed at shorter time intervals. Rats prepared in the same way as above were killed at 4 and 8 hr., as well as at 24 hr., after ³²P was administered. In order to reduce the amount of work involved, we studied only three dietary groups, namely animals receiving the protein-free diet at low and at high levels of energy intake, and animals

Table 4. The effect of changes in energy intake (carbohydrate) on the uptake of ³²P by liver ribonucleic acid at 4, 8 and 24 hr. after injection of labelled P

(Each figure is the mean of observations made on four rats.)

Time after ³² P injection (hr.)	Type of diet	Mean daily energy intake (kg.cal./sq.m.)	Mean initial body weight (g.)	Specific activity of inorganic P of liver (counts/min./100 µg. P)	Relative specific activity of acid-soluble organic P	Relative specific activity of nucleotides			
						Whole RNAP*	Adenylic and cytidylic acids	Guanylic acid	Uridylic acid
4	Protein-free	870	179	4860	84	7.5	7.6	5.2	8.7
	Protein-free	1880	179	4714	85	9.8	9.4	8.2	11.3
	Protein-containing	1840	181	3638	—	4.4	—	—	—
8	Protein-free	950	177	3063	102	17.0	16.9	14.1	18.5
	Protein-free	1850	182	2419	110	19.2	19.1	17.5	21.3
	Protein-containing	1840	186	2234	93	13.1	12.6	10.2	16.3
24	Protein-free	840	186	1769	105	34.1	33.1	32.5	34.6
	Protein-free	1890	173	2237	104	54.2	51.6	51.6	54.1
	Protein-containing	1725	184	1808	100	32.1	31.2	28.6	34.2

* Short run ionophoresis, with nucleotides eluted together.

receiving the protein-containing diet at the higher plane of energy intake. These three groups provide all the necessary contrasts (cf. Table 3). In all three groups the specific activity of liver inorganic phosphate showed a similar trend with time (Table 4). The relative specific activities of RNAP from animals receiving the protein-free diet at the higher plane of energy intake were the greatest at all time intervals after injection. In addition, this experiment indicates the behaviour of individual ribonucleotides obtained by prolonging the period of ionophoresis. Guanylic and uridylic acids were completely separated, but it was found necessary to elute adenylic and cytidylic acids together. It is apparent (Table 4) that, under the same conditions of diet and time after isotope injection, the various nucleotides incorporate ^{32}P to essentially the same extent. Each nucleotide shows a higher incorporation rate when obtained from animals on the protein-free diet at the higher level of energy intake. This would appear to eliminate the possibility that contaminants play a significant part in accounting for the differences in phosphorus incorporation into RNA, since they could hardly affect all nucleotide fractions similarly. A further feature of this experiment is the behaviour of the acid-soluble organic phosphates of the liver (Table 4). The specific activity of this fraction is not significantly altered by the nature of the diet at any of the time intervals after injection of the labelled phosphate. This suggests that the changes displayed by RNA phosphorus in relation to diet are not shared by phosphorylation in general.

The experiments so far described refer to changes in energy intake produced by alterations in dietary carbohydrate. The effect of adding fat to a protein-free diet has also been determined. In these experiments one series of rats was maintained at an energy intake of 1200 kg.cal./sq.m. by adding carbohydrate to a protein-free diet and another series by adding fat; after a week at this level, different energy intakes were obtained in each series by varying the

amount of carbohydrate or fat fed. Table 5 shows that the addition of fat was at least as effective as the addition of carbohydrate in stimulating the rate of incorporation of ^{32}P into RNA. An additional experiment with fat has confirmed this picture.

Table 5. *Comparison of the effect on ^{32}P uptake by liver ribonucleic acid of adding fat or carbohydrate to a protein-free diet*

(Groups of three rats.)		
Source of energy variation	Daily energy intake (kg.cal./sq.m.)	Relative specific activity of RNAP
Carbohydrate	1000	25.7
	1920	32.4
Fat	980	23.2
	1900	30.5

Analysis of variance shows that the influence of energy intake is significant ($P < 0.05$) but that the action of carbohydrate does not differ from that of fat (interaction not significant).

Two final experiments were performed in an attempt to throw some light on the mechanism underlying the changes in RNAP synthesis observed in the preceding experiments; they differ slightly from the foregoing experiments in the details of the diets fed. In the first of these additional experiments, rats were fed either a protein-containing or a protein-free diet at a high level of energy intake for a week, and then each was injected with labelled phosphate. Thereafter, half of each dietary group were given glucose *ad lib.* and the rest were fasted. All animals were killed 24 hr. after injection. The data show (Table 6) that the rate of ^{32}P incorporation was determined by the previous diet and not by the immediate ingestion of glucose. Thus the group on the protein-free diet did not lose their higher incorporation rate when fasted 24 hr., and the high-protein group did not exhibit an increased incorporation rate when fed for 1 day exclusively on glucose. This picture is amplified by

Table 6. *The effect of glucose administration during a 24 hr. period following high- and low-protein diets on total ribonucleic acid/liver and the rate of ^{32}P incorporation into ribonucleic acid*

(Groups of four rats.)					
Diet	Initial body weight (g.)	Daily energy intake on diet (kg.cal./sq.m.)		Total RNAP/liver (mg.)	Relative specific activity of RNAP
			Food during final 24 hr.		
Protein-containing	226	1680	Glucose	5.09	39.9
	230	1680	None	4.65	36.7
Protein-free	233	1705	Glucose	4.22	48.2
	230	1725	None	4.56	53.2

Analysis of variance shows that the amount of RNAP in the liver is significantly affected ($P < 0.05$) by the administration of glucose during the final 24 hr., when the preceding diet contained protein. There is a highly significant difference ($P < 0.01$) in relative specific activity at the two protein levels, but no significant effect of glucose at either protein level.

the final experiment (Fig. 3). In this case, all animals were first fed a diet providing an adequate amount of protein and energy (1700 kg.cal./sq.m.).

Fig. 3. The influence of protein intake on the amount of ribonucleic acid in the liver (upper two curves) and its uptake of radioactive phosphorus (lower two curves). After a week on an adequate diet, the rats were given either a diet containing protein (●—●) or a similar diet free from protein (○---○), both diets being fed at a level of 1700 kg.cal./sq.m. body surface/day. The animals were injected with ³²P as orthophosphate 24 hr. before killing. Each point is the mean of observations on three rats.

After they had been conditioned to this diet for a week, half the animals were given a protein-free diet of equicaloric value, carbohydrate being substituted for protein. Thereafter, groups of three

rats from each diet were killed daily, having been injected 24 hr. previously with labelled phosphate. The transfer to the diet lacking protein caused an immediate reduction in the RNAP/liver but did not induce an immediate rise in the rate of ³²P incorporation. In fact there was first a fall, followed by the expected higher level from the second day onwards. The rats which continued to receive the protein-containing diet (controls) showed a constant amount of RNAP/liver and no significant alteration in the rate of ³²P incorporation. According to Student's 't' test (Snedecor, 1946), both the initial reduction in ³²P incorporation rate on the first day of the protein-free diet and the subsequent increase in rate differ significantly (*P* < 0.01) from the rate of incorporation by the control rats killed on the same day.

DISCUSSION

The above experiments indicate that energy intake influences the metabolism of RNA. In order to compare the magnitude of the alterations in amount and in rate of incorporation of ³²P caused by variations in energy supply, we have calculated the change produced by an increment of 1000 kg.cal./sq.m. of body surface area and have expressed it as a percentage of the amount or the incorporation rate obtaining at an energy intake of 1200 kg.cal./sq.m. In Table 7, all experiments involving a change in energy intake from carbohydrate have been set out in this way (experiments with fat are fewer, but show the same general trends). With regard to changes in total RNA/liver, we have not only the results obtained by the orthodox Schmidt-Thannhauser procedure, but also more accurate estimates of the amount of RNA obtained in the course of the radioactivity experiments by ionophoretic separation of the ribonucleotides. Table 7 shows that, when the diet

Table 7. Comparison of the changes in total ribonucleic acid/liver and ³²P uptake by ribonucleic acid when energy intake from carbohydrate is increased

(Regression coefficients indicate the change produced by adding 1000 kg.cal./sq.m. body surface area to the diet, and have been expressed as a percentage of the amount of RNAP or the relative specific activity of RNAP corresponding to an energy intake of 1200 kg.cal. Figures in brackets are the number of animals in each experiment.)

Diet	Data recorded in	Method of RNA isolation	Regression coefficients	
			Total RNAP/liver (%)	Relative specific activity of RNAP (%)
Protein-containing	Fig. 1	Schmidt-Thannhauser	+ 29.2 (16)	—
	Table 1	Schmidt-Thannhauser	+ 20.8 (20)	—
	—	Ionophoresis	+ 28.6 (24)	+ 3.4 (16)
Protein-free	Fig. 1	Schmidt-Thannhauser	+ 10.9 (16)	—
	Tables 1 and 2*	Schmidt-Thannhauser	+ 4.4 (24)	—
	—	Ionophoresis	+ 8.1 (57)	+ 26.5 (33)
	Fig. 2	Ionophoresis	—	+ 27.8 (12)

* The animals shown in Table 1 as receiving a protein-free diet include most of those given in Table 2 (carbohydrate added).

provides adequate amounts of protein, an increase in energy intake of 1000 kg.cal. causes a rise of some 20–30 % in total RNAP/liver, without significantly altering the proportion of P atoms, replaced in a given time. With a protein-free diet, the total amount of RNAP is only slightly increased (about 5–10 %) when the intake of energy is raised by 1000 kg.cal., but the rate of replacement of P atoms is augmented by about 25 %. It is thus apparent that, at each level of protein intake, the total number of P atoms incorporated into RNA is increased by raising the intake of energy, in one case by an increase in total RNAP/liver without a change in the percentage of P atoms incorporated in a given time, in the other case by an increase in incorporation rate with a smaller change in total RNAP/liver. It is clear from Table 7 that the magnitude of the change in incorporation rate on the protein-free diet is fully adequate to compensate for the lack of alteration in total amount. This can also be demonstrated more directly by calculations based on the data given in Table 3. By multiplying the specific activity of the RNAP by total RNAP/liver, we obtain a measure of the total amount of ^{32}P incorporated per liver. This has been done in Table 3 (last column), the figures being adjusted by covariance analysis for differences in the specific activity of the inorganic phosphate of the liver. It will be seen that at each level of protein intake, a rise in energy intake is accompanied by an increased absolute incorporation of ^{32}P . The effect of energy intake on ^{32}P incorporation is not significantly different at the two levels of protein intake.

It is thus clear that two dietary factors influence the course of RNA metabolism in the liver. Protein intake plays a dominant role in determining the amount of RNA in the liver, a finding in agreement with that of Campbell & Kosterlitz (1950, 1952), whereas energy intake determines the absolute rate of phosphorus incorporation. This explains why the unrestricted feeding of a protein-free diet results in a reduction in liver RNA accompanied by a compensatory rise in the turnover of phosphorus in the remaining molecules (Fig. 3). Campbell & Kosterlitz (1948, 1952) were the first to draw attention to this phenomenon, although they were unaware of its explanation.

The observation that energy intake determines the absolute rate of ^{32}P incorporation into RNA raises the question of whether this reflects real differences in the rate of RNA synthesis or whether the phosphorus is incorporated by an exchange reaction. Decisive evidence can only be obtained by studying incorporation of isotopes into other parts of the nucleotide structure, but it should be pointed out that, in view of the commonly accepted structure of RNA, it seems unlikely that most of the P atoms can be exchanged with the medium except by

molecular resynthesis. Even if we accept the data as representing the behaviour of the whole RNA molecule, the significance of the findings remains obscure. In this connexion two of our experiments present suggestive features. Changes in energy intake do not immediately affect the rate of ^{32}P incorporation, as shown by the experiment in which rats receiving a protein-free diet with abundant energy intake were either fasted or fed glucose shortly before death (Table 6). Furthermore, in contrast to the findings of Campbell & Kosterlitz (1948) with unpurified RNA preparations, removal of protein from the diet does not result in an increased uptake of ^{32}P by RNA until the level of RNA in the liver has ceased to fall (Fig. 3). This suggests that, during the first day of the protein-free diet, the needs of the liver cell are met by the considerable breakdown of RNA attached to protein. Thereafter, the requirements of the cell can only be provided by increasing the percentage turnover of RNA. The nature of these requirements is a matter for future investigation. It seems unlikely that they can be explained in relation to protein synthesis, since increments in energy intake do not increase the amount of protein in the liver when the animals are receiving a protein-free diet (Munro & Naismith, 1953), whereas RNA synthesis is increased under these circumstances. This point can, however, only be finally decided by simultaneous study of the rates of RNA and protein synthesis on protein-free diets. Finally, there is the possibility that RNA serves as a source of nucleotides for coenzyme synthesis, in which case it would not be unnatural to find that the requirements of the cell for RNA were proportional to energy intake.

SUMMARY

1. A study has been made of the effect of variations in energy intake on the amount of ribonucleic acid (RNA) in the rat liver and on the uptake of labelled phosphorus by RNA.
2. When the basal (sub-maintenance) diet contained protein, addition of energy in the form of either carbohydrate or fat resulted in a considerable increase in total RNA per liver; when the diet lacked protein, an increase in energy intake caused only minimal changes in the amount of RNA.
3. The uptake of ^{32}P by RNA, as measured by relative specific activity, behaved in the opposite way. Uptake was unaffected by variations in energy intake when the diet contained protein, but it was considerably stimulated by addition of energy to the protein-free diet.
4. These results indicate that the absolute rate of incorporation of phosphorus into RNA is dependent on energy intake. At each level of protein intake, the total number of phosphorus atoms incor-

porated into RNA is increased by raising energy intake, in one case by an increase in the total RNA per liver without a change in the percentage of phosphorus atoms incorporated in a given time, in the other case by an increase in percentage incorporation rate accompanied by a smaller change in total RNA per liver.

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Biochemical Changes Occurring During the Germination of Bacterial Spores

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Spores of laboratory strains of *Bacillus subtilis* and *Bacillus megatherium*, incubated with L-alanine and glucose respectively, remain viable, but rapidly lose their resistance to heat (Hills, 1950; Powell, 1951). They also become less refractile and more permeable to stains (Powell, 1951). These changes indicate the first stages of spore germination and are accompanied by a decrease in cell dry weight and an increase in total and amino nitrogen of the suspending medium. It appears that excretion is one of the first changes associated with the germination process, whether this occurs in a medium containing only the specific germination stimulant or in a nutrient tryptic digest broth (T.M.B.), or spontaneously in water suspension as a result of prolonged heat activation. This excretion process and the identification of the excreted solids will be described in detail.

ORGANISMS AND METHODS

Most of the work was done with spores of the laboratory strain of *B. megatherium*. These were obtained by growth on meat extract peptone agar containing 0.2% Lab Lemco, 1% peptone, 0.5% NaCl, and 0.2% glucose (Tarr, 1933). They were reaped after 2 days' growth at 37° and washed at least five times with water. During reaping and the first

three washings, suspensions were cooled to 0°, since it had been previously found that some incompletely washed suspensions germinated spontaneously at room temperature. Spores of this organism need glucose specifically for germination and, in some cases, a short preliminary heat treatment, i.e. 30 min. at 60° (Powell, 1951). It has been recently found that if a prolonged heat treatment is given, i.e. 2 hr. at 60°, and the suspension is then centrifuged and resuspended in water, spontaneous germination takes place. Within an hour, more than 90% of the spores lose their heat resistance and stain densely, but are still viable. These changes occur in suspensions as thick as 1.5×10^{10} spores/ml. Germination exudates were obtained in this way, and also by incubating resting spores with 5 mM-glucose and 30 mM-phosphate buffer, pH 7.3. After 30–45 min. incubation the suspension was centrifuged, and the clear and colourless centrifugate removed for analysis.

A less complete investigation was made with spores of the laboratory *B. subtilis*. These were obtained after 3–4 days' growth at 37° on casein-yeast (CCY) agar (Gladstone & Fildes, 1940). They were washed five times with water, and had no tendency to germinate spontaneously during reaping or after heat treatment. In 30 mM-phosphate with 2 mM-L-alanine and 5 mM-glucose, germination was generally not more than 30–50% in suspensions of 5×10^8 spores/ml., and could only be improved by the addition of disproportionately high concentrations of L-alanine which then obscured subsequent analyses and amino-acid chromatograms. Germination was almost complete in suspensions