in young mammalian embryos (rabbit, sheep, pig, guinea pig, and human) erythrocytes develop directly from hepatic parenchymal cells. These recently recorded results, however, lack confirmation, and the supporting illustrations admit of an alternative interpretation.

It is well known that in pernicious anemia generally the red corpuscles are of larger average size, thus determining a color index above unity, and that megaloblasts (initial stages in red cell formation) occur; but the relatively larger size of both may be quite as well, and much more likely, the result of an absence of normal proliferative activity of these proerythroblasts, as of persistent entodermal ancestors. The familial incidence of pernicious anemia may equally plausibly be explainable on the basis of a hereditary susceptibility to factors that affect the bone marrow, and other possible erythrocytopoietic foci, in such a way as to produce a condition characterized by relatively low proliferative activity among the ancestral cells, resulting in the appearance of many relatively large red cells, both mature and immature.

Another interpretative conclusion, in conflict with practically all recent work, relates to the restricted ancestry of the monocytes. According to Piney, monocytes are derived from the reticulo-endothelial system exclusively through the myeloblasts, a monocyte being "as much a myeloid cell as is any other granular leukocyte."

Though emphasizing the alleged distinctive character and apparent independence of the lymphatic and myeloid tissues in many respects, Piney is forced to admit certain obvious interrelationships as indicated in certain infections where lymphocytosis coexists with a neutrophilia, following a leucopenia during the height of the infective process. A probable explanation seems to be at hand in the repeated demonstrations that lymphocytes may develop into granulocytes. At the height of the demand for neutrophils the lymphocytes may be very rapidly converted, effecting a resulting lymphocytopenia, which may be later overcorrected through an attempt at compensatory readjustment of normal numerical relationships. Similarly the anemia almost invariably accompanying leucemia might be interpreted in terms of the limited availability of the common ancestor (the lymphoid hemoblast) for erythrocytes, granulocytes and monocytes. When the specific stimuli are such as to demand an excess of one of the derivatives, the other possible differentiation products may be reciprocally, at least temporarily, reduced in numerical proportion. This differs from Piney's explanation that since (as he claims) erythrocytes arise only intravascularly, and leucocytes extravascularly, specific morbid stimuli may reach one surface of the common blood cell primordium without affecting the opposite surface.

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DOES THE AMOUNT OF FOOD CONSUMED INFLUENCE THE GROWTH OF AN ANIMAL?

THE question to which this discussion is addressed might well be considered an impertinent one to raise in a scientific journal were it not for the fact that a number of research laboratories in this and other countries appear to be attacking many important problems in nutrition by methods involving the tacit assumption that *the composition of the diet alone* determines the changes in body weight secured in experimental animals. When stated in such plain terms as these, the situation, if it actually exists, would appear to be sufficiently serious to justify general consideration and discussion, to the purpose that the time and energy being devoted to nutritional problems should yield the greatest return in unequivocal experimental evidence.

Obviously, the point of first importance is to determine whether the situation characterized above actually exists. This characterization may be illustrated in an abstract way as follows. It is desired to determine whether a given food material is deficient in a certain dietary essential A. Accordingly, two rations are made up, including the food material in question supplemental with adequate percentages of all known dietary essentials except A. In one ration, A is included in place of an equal percentage of a dietary diluent such as starch, while in the other ration the food itself serves as the sole source of A. Now, it is the general plan to feed ration No. 1 to one group of animals, and ration No. 2 to another, the food in each case being offered *ad libitum*. The changes in weight of the animals are then followed by periodical weighings. In some cases, records of food consumed may be kept and, in fewer cases, such records may be reported, but in the great preponderance of cases, no *control* of food intake is imposed, and the weight curves are interpreted with reference only to the composition of the rations.

Is not this type of interpretation tantamount to assuming that the amount of food consumed has no influence on growth? The assumption itself is obviously untenable, and the interpretation can be defended only on the very tenuous supposition that the

more adequate of two rations will *always* be consumed in the larger amount and that, therefore, a greater consumption of one of the two comparable rations is, itself, *prima facie* evidence of its nutritive superiority over the other. While it is true that there is a marked tendency, frequently referred to in the literature, for experimental animals to eat sparingly of markedly deficient diets,[1] to assume that this is the only factor of importance operating in the determination of the daily consumption of food is to deny the influence of flavor, odor, and texture of the diet, and to endow the appetite of the animal with an infallibility impossible to explain on physiological grounds or to believe on empirical grounds.

Returning to the hypothetical illustration, if ration No. 1 has induced better growth than ration No. 2, there are two possible explanations, either (a) it is actually superior in its nutritive balance, so that if consumed in amounts no greater than ration No. 1, it would still induce better growth, or (b) it has been consumed in so much larger amounts than ration No. 2 that better growth has resulted from this fact alone, with no reference to the difference in composition. Current practice neglects the latter possibility entirely, apparently because no obvious reason exists for supposing that ration No. 1 is more palatable than ration No. 2; hence, if it has been consumed in larger amounts it must be because it is more nearly adequate in nutritive value. But this is devious reasoning. It is true in many cases it may lead to a highly probable conclusion; nevertheless, in no case can an interpretation based upon such reasoning be awarded the finality of a demonstration. Surely it is the purpose of experimentation to demonstrate something whenever possible, rather than to set up a certain probability of its truth, and in this type of work a demonstration can be secured only by controlling the food consumption of the experimental animals in such a way that the intake of food by comparable groups is the same, either absolutely or in proportion to their requirements.

The many different problems in which this question of experimental technic is involved can only be appreciated by specific illustrations. The significance of food intakes is involved in every step of the method for the "biological analysis" of foods introduced into rather general use about twelve years ago, and the neglect of dietary control with reference to the amount of food consumed is a proper obstacle to the acceptance *in toto* of the deductions regarding the dietary deficiencies of the large number of foods investigated. The method has contributed valuable viewpoints to the science of nutrition and has undoubtedly furnished an essentially accurate bird's-eye view of the situation with reference to relative food values. Nevertheless, the details of the results with reference to any particular food can not be considered as established facts until confirmed by more refined methods. As a pioneer method, it has served its purpose well, but pioneer methods are of use only in pointing the way to profitable investigation by providing working hypotheses; it is unfortunately true that several laboratories are still using the method in a routine way in attacking specific problems.

A recent contribution from an agricultural experiment station, involving several years' work upon a large number of animals and rations, attempts a scientific analysis of the factors concerned in the production of rickets (posterior paralysis) in swine with no reference at all to the food records secured, though these records were extremely variable among the lots of animals compared. If the efficiency of a number of rations in promoting growth or protecting against disease varies in close correlation with the amounts of them consumed by the experimental animals, it is but natural to suppose that the biological reactions observed may have been determined entirely by the intake of food regardless of its composition. If one of two comparable rations has induced a more rapid growth than the other, as well as a greater consumption of food, there must always be a doubt as to the growth that would have resulted from the other ration if it had been consumed in as large amounts as the first.

Fortunately, the ambiguity inherent in the experimental results obtained from the "biological analysis" of a food by means of *ad libitum* feeding experiments is becoming increasingly evident to investigators in nutrition. The prevailing tendency in determining the nutritive value of a food material is to consider each individual nutrient as presenting a problem in itself, to be investigated by a method found to be most effective for that particular nutrient. As a result, there are available at the present time reasonably accurate methods for the determination of the protein values of foods and of the relative concentrations of foods in vitamins. These methods differ essentially among themselves in the experimental conditions imposed, the kinds of animals used, and their preliminary preparation. In the most accurate methods for the quantitative study of the distribution of vitamins, the necessity of securing definite intakes of the food material under investigation is fully recognized, although the intake of the basal ration used in providing a suffi-

[1] The conclusion has also been drawn that rats eat in accordance with their energy requirements rather than in accordance with the balance of nutrients in the ration. Furthermore, many instances have been reported in the literature in which inadequate diets are consumed for a considerable time in adequate amounts, and vice versa.

cient source of energy and in supplementing the food in all essential dietary factors except the one under investigation, is not controlled. It may be questioned whether control of the intake of basal ration is not also advisable, since it will undoubtedly vary with different animals, and will be inversely correlated with the intake of the food material being studied. For the same intake of the food under examination, a variable intake of basal ration presumably would affect the rate of growth secured.

Much work has been done upon the existence of vitamins specifically concerned with reproduction and with lactation. Feeding experiments with uncontrolled food consumption have been used throughout this work, and several of the conclusions drawn from such experiments seem unwarranted because of the failure to realize that the biological performance of an animal may be distinctly different on different intakes of food, particularly if the rations fed are not absolutely deficient in any essential food factor. The confusion prevailing in this field at the present time and the slow progress being made may be considered as an inevitable result of improperly controlled experimental procedures.

The question of the significance of arginine and histidine in nutrition has occupied the attention of a number of investigators, in this country and in England, during the last ten years. While it is the consensus of opinion that the indispensability of histidine is well established, the evidence is conflicting with reference to arginine. Again the trouble appears to be related to the methods of experimentation, *i.e.*, feeding experiments on rats in which the food intake is uncontrolled. One laboratory reports that the addition of either arginine or histidine to an amino acid mixture deficient in both renders it capable of supporting growth, a result indicating an interchangeablility of these two amino acids in metabolism. Another laboratory has reported success in this experiment with histidine only, repeated attempts to supplement with arginine resulting invariably in failure to secure growth, though long-continued maintenance of weight was occasionally observed. This laboratory most emphatically denies the interchangeability of arginine and histidine on the basis of its negative results. Other laboratories have reported favorable effects of arginine additions to amino acid mixtures deficient in both arginine and histidine, though only maintenance of weight rather than growth resulted. In those experiments in which food intakes are reported, it is a significant observation that where failure of growth and of maintenance resulted on the arginine rations, the food intakes were so low that it is a fair suspicion that they were inadequate for the maintenance of weight. In fact, the results of this series of experiments suggest that success in obtaining growth on the arginine rations was in proportion to the success in inducing the animals to consume them.

It is perhaps a sound criterion that a ration can not be adjudged incomplete or inadequately balanced, until nutritional failure results *when it is consumed in adequate amounts,* or unless it induces distinctly less favorable results in experimental animals than a ration known to be complete, *when they are consumed in equal amounts.*[2] Under the circumstances, therefore, more significance would appear to attach to the favorable results obtained by arginine additions than to the unfavorable results. It is undoubtedly desirable that the interchangeability of arginine and histidine in metabolism be confirmed if possible, preferably by some other method more amenable to interpretation; nevertheless, it is a fair conclusion that the negative results reported do not disprove the possibility of an interchangeable relation.

A natural extension of such inadequately controlled feeding experiments to the solution of problems of intermediary metabolism has been in evidence during the last few years. The question whether taurine can replace cystine in metabolism has been studied in four laboratories by feeding experiments with small animals. In one laboratory the results obtained were taken to indicate the possibility of such a substitution; in two laboratories the non-committal conclusion was drawn that no evidence of such a possibility was obtained; while in one laboratory the negative evidence secured was the basis of the conclusion that taurine is "totally incapable" of replacing cystine in the diet for purposes of growth. However, the lack of control of food consumption in all these experiments in assuring comparable food intakes among otherwise comparable groups, renders a definite interpretation of them difficult, if not impossible.

Similarly, the results of experiments involving rations containing, in place of histidine, imidazole derivatives and other compounds with which it might be related biochemically, have been interpreted with reference to the possibility or otherwise of certain metabolic reactions involving this amino acid. No criticism can be made of the most obvious interpretation of such experiments when continued growth is observed; but when failure of growth results, interpretation seems impossible, although it is commonly supposed to demonstrate that the ration used is inadequate. This, however, appears to represent an

[2] As proof that this is not an impossible or impracticable requirement, the demonstration that non-leucine can not replace lysine in metabolism may be cited (Lewis and Root, J. Biol. Chem., 1920, xliii, 79).

exaggeration of the importance of negative experimental results.

Negative experimental results may have two meanings: (1) They may demonstrate definitely that something is not true, and, if this outcome is quite unexpected in view of contemporaneous theories and conceptions, the results may be of great significance. On the other hand, (2) they may represent simply unsuccessful attempts to demonstrate something, due to the use of inadequate experimental methods or to the selection of unsuitable experimental conditions, in which case they are of no considerable importance. It would appear that the type of negative experimental result obtained with inadequately controlled feeding experiments should be classified under (2), since it has not been definitely proven by proper control experiments that the failure of growth or maintenance can *only* be due to the inadequate composition of the ration.

As illustrating the ultimate result of a line of reasoning that is so frequently implied and pursued in the interpretation of incompletely controlled feeding experiments, another recent investigation concerned with histidine synthesis in the animal body may be cited. In this study evidence was obtained of the successful substitution of histidine by imidazole lactic acid and also by imidazole pyruvic acid; and a distinctly favorable effect on body weight of the addition of imidazole acrylic acid to histidine-poor rations was also observed. From the rates of growth secured on the different rations, conclusions are deduced concerning the intermediary metabolites of histidine, the reversibility of the reactions involved, and even the relative speeds of such reactions. These interpretations fail to consider the great differences observed in the consumption of the different experimental rations, which have been averaged and summarized in the following table:

No. of periods averaged	Rations	Aver. body weight of rats gms.	Aver. daily food intake gms.	Aver. daily gain in weight gms.
17	Basal, no histidine	99	2.3	−0.86
6	Basal, plus histidine	107	7.2	+1.67
4	Basal plus imid. lactic acid	98	5.9	+1.36
4	Basal plus imid. pyruvic acid	81	3.8	+0.45
6	Basal, plus imid. acrylic acid	112	3.2	−0.10
3	Basal plus imidazole	102	3.4	−0.25

It is evident that the changes in weight, although obtained in prompt response to the changes in ration, are closely correlated with the amounts of food consumed, and hence, in the absence of any evidence to the contrary, may be the direct result of the variable food intake. The experiments can not be said to demonstrate that the basal ration was inadequate, since the daily consumption of 2.3 gms. of food, no matter how well-balanced it may be, could not maintain the weight of a 100 gm. rat. The responses to the various additions to the basal diet may indicate: either (1) that the basal ration is in fact adequate and is capable of supporting growth when consumed in adequate amounts; or (2) that the basal ration is inadequate but that all of the imidazole derivatives added to it are equally effective substitutes for histidine, the variable growth secured depending primarily upon the amount of food consumed: or, finally (3) that the basal ration is inadequate and that the various additions to it are effective in replacing histidine in metabolism in proportion to the amount of growth secured, the variable food intakes being a purely incidental manifestation of the infallibility of the animal appetite in consuming food in proportion to its completeness in meeting the body's requirements. The authors of this report subscribe to the third interpretation, without, however, considering or refuting the other two.

These illustrations of published investigations involving feeding experiments on animals may be taken as proving that biological investigations are frequently based upon the implicit assumption that the amount of food an animal consumes has no effect upon growth and hence is not a legitimate part of the experimental evidence and, therefore, may be disregarded in the interpretation of results; or upon the equally erroneous assumption that the amount of food an animal consumes is determined solely by its value in nutrition. An experimental animal is considered as a sort of biological reagent of marvelous accuracy and varied uses, capable of giving as significant a response in body weight change to a change in ration, as the color response of a chemical indicator to a change in hydrogen ion concentration around the point of neutrality. Unfortunately this conception of the infallibility of experimental animals can not go unchallenged, and the methods of experimentation blindly involving this conception are in need of revision. They can not be considered to be properly controlled except when the rations whose nutritive effect it is desired to compare are fed in equal amounts to comparable animals. Even when the amounts thus fed are inadequate, due to the refusal of the experimental animals to consume readily one or both of the rations, it is reasonable to expect that the inferior ration will ultimately induce a greater decline in

weight than the other, or that it will lead to total nutritive collapse sooner. Admittedly experimentation under these conditions is not wholly satisfactory, but investigators should reconcile themselves to the fact that it is difficult to demonstrate the inadequacy of a given ration in any essential dietary factor when the experimental animal will not readily partake of it. Probably in many situations of this character the use of growth experiments is contraindicated. The success of any biological investigation is undoubtedly endangered when the experimental animal will not cooperate to a certain minimum extent.

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SPECIAL ARTICLES

OVARIAN SECRETION AND TUMOR INCIDENCE

FOR several years an attempt has been made to build up, by selective inbreeding, lines of mice which should have a very high incidence of mammary cancer. This effort has been quite successful, and it has been possible to establish two lines in an inbred dilute brown stock,[1] which produce very nearly 100 per cent. cancerous females. Among 183 breeding females of this dilute brown stock, taken in linear order from our ledger, 122 were tumorous. Of the remaining sixty-one, many died before reaching tumor age.

Under normal breeding conditions the neoplasms appear in the vicinity of the mammae of the females between the ages of four and fourteen months. The tumors usually appear, however, between the beginning of the seventh and the end of the eleventh months, the mode being at nine months.

It has been known for some time that the internal secretions of the ovaries play an important part in the physiological condition of females during and after the gestation period. That the influence of these hormones has also a direct effect upon the ability of mice to combat the growth of neoplasms has been demonstrated by Dr. L. C. Strong[2] (1922) in his work upon transplanted tumors. Dr. Leo Loeb[3] has also published (1915) a brief note on the effects of castration and enforced non-breeding on tumor incidences. More recently (1927) Dr. Carl F. Cori[4] has published a very interesting paper on the results of castration and ovarian transplantation in mice.

[1] This stock has been developed from a single pair by Dr. C. C. Little, and has been inbred, for the most part, by brother to sister matings since 1909.

[2] Strong, L. C., *Jour. Expt. Zool.*, 36: 1, 1922.

[3] Loeb, L., SCIENCE, Vol. XLII, No. 1095, Dec. 24, 1915.

[4] Cori, Carl F., *Jour. Expt. Med.*, Vol. XIV, No. 6, June 1, 1927, pp. 983–991.

I. What is the effect of enforced non-breeding upon cancer incidence in female mice?

That either or both of two factors: (a) the stimulating effect of lactation and pregnancy, and (b) the effect of sex hormones, may be involved in the appearance of cancer in the stock under observation, is demonstrated by the fact that when 207 virgin females of the dilute brown stock were separated from the males before sexual maturity and allowed to grow old under exactly the same conditions as the stock mice, which in many cases were siblings of the virgin females, but twenty tumors have appeared among them, although the youngest of these animals is fifteen and a half months old. The earliest age at which cancer appeared was ten months, the average 14.7 months, and the oldest seventeen months, as contrasted with four months, nine months and fourteen months for the breeding females (see Table I, lines 1 and 2).

TABLE I

AGE IN MONTHS

	Youngest	Average	Oldest
Breeding females	4	9	14
Non-breeding females	10	14.7	17
Castrated females	9	15.6	18.8
Castrated males with ovarian implants	8	11	13

From this we may infer that enforced non-breeding delays very markedly the age of tumor appearance and may even inhibit entirely the development of cancer in mice, which would probably have had a high incidence of tumor appearance had they lived a normal sexual life.

II. Will the female mouse grow a tumor when completely castrated?

The ages at which tumors occur in the normal breeding females indicate that the appearance of the neoplasms is closely correlated with the normal[5] activity of the ovary and ovarian hormones. One possibility is that cessation of ovarian action following a period of activity is the chief stimulating factor in producing mammary cancer. If, then, females in which the ovary has functioned at a low rate for a time are completely castrated, it might be expected that these animals will develop tumors at an age which is actually *younger* than that at which normal breeding females develop cancer. With this in mind, 210 females were spayed and allowed to grow old under the same laboratory conditions as the stock animals. To date twenty-one of these females have developed

[5] Normal activity of the ovary is here taken to mean that of a breeding female rather than a non-breeding animal.