Our results substantiate Paul's prediction (*1*) that media intended for mammalian cells would probably suffice for in vitro cultivation of cold-blooded animal tissue.

Cells of all the animals used grew in a medium consisting of 10 percent human cord serum (2 to 5 percent serum was suboptimal; a 20-percent level was unnecessary); 5 percent whole egg ultrafiltrate (chick embryo extract was contraindicated); 30 percent medium 199, 0.5 percent lactalbumin hydrolyzate, 10 percent Hanks' balanced salt solution, 45 percent Earle's balanced salt solution (which contained one-half the prescribed $NaHCO_3$), and antibiotics (400 units each of penicillin and streptomycin and 50 units of nystatin per milliliter). Melnick's monkey-kidney medium A or B, with an additional 8 percent calf serum, was adequate for initial attachment and growth of rainbow trout cells but was not tried on cells of other animals.

Soft-organ tissue was used in all trials, and a variety of results was obtained. Attachment and division of cells from testes of mature trout was consistently poor. Except for a largemouth bass (*Micropterus salmoides*), whose ovaries were infested with cestodes, ovarian or pooled immature gonadal cells of the animals tested readily attached and divided; good results were obtained for painted turtle (*Chrysemys picta*), rainbow trout (*Salmo gairdneri*), eastern brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), bluegill (*Lepomis macrochirus*), and goldfish (*Carassius auratus*). Mature reproductive tissues of the bullfrog (*Rana catesbeiana*) gave poor results, but the kidney and to a lesser degree the heart cells were readily cultivated. In work with the rainbow trout, excellent monolayers were prepared from larvae. There was fair attachment and some mitosis among cells of the swim bladder, spleen, and kidney of the adult rainbow trout, but liver, heart, intestine, and gills gave poor results.

Initial attachment of cells of all animals began almost immediately, even in the hemocytometer. Depending upon initial density, cell dispersions from reproductive tissue of fish gave uniform monolayers of epithelial-like and spindle cells in 1 to 6 days at 19°C (Fig. 1). Monolayers of frog and turtle cells formed more slowly.

Subcultures of fish cells have been prepared also by mechanical dispersion, by a 10-minute cold digestion with 0.25-percent trypsin, but preferably by 10-minute cold dispersion with disodium versenate (20 mg/100 ml) followed by immediate "neutralization" with the medium in which the cells had been grown.

Subcultures of fish cells have been carried on the media described, on the growth medium of Puck *et al.* (*9*), and on media consisting of 10 percent human cord serum and either 90 percent Eagle's basal medium, Eagle's minimal essential medium, or NCTC-109.

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18 August 1960

Hormology in Nutrition

Abstract. Discrepancies in the growth curves for the sodium requirement of the cricket indicate that hormology may play an important role in the response to essential nutrients as well as to toxic compounds. Differentiation between stimulation of growth by a nonnutritive action and support of growth by an essential nutrient is difficult.

Discrepancies in data obtained during the determination of the quantitative requirement for sodium in crickets (*1*) suggested the possibility that this and other unexplained phenomena in nutrition may be due in part to hormology. Hormology has been defined as the study of stimulation and excitation (*2*). The general term includes hormoligosis, the stimulation resulting from any harmful agent present in minute quantities. Hormones are presently being studied as typical systems of hormology. Hormetics (*3*) are toxic compounds which excite in low concentrations. Many reactions of pharmacology show typical dose-response curves predicted by hormoligosis (*4*).

Multiphasic response curves were obtained by Richet (*5*) with concentrations of heavy metals which were 10 to 1000 times less than those which exhibit the harmful oligodynamic action reported by Nägeli (*6*). Schultz (*7*) and Branham (*8*) have shown that most of the classical bactericidal compounds stimulate bacteria when present in very dilute concentrations. Antibiotics sometimes exhibit this phenomenon. The myriad reactions reported for antibiotics in nutrition (*9*) cannot all be attributed to the intestinal microorganisms—for example, the decreased mineral requirement, or the changes seen in germfree plants (*10*) and animals (*11*). Other unexplained phenomena in nutrition include (i) the observation that certain derivatives of vitamin B_{12} are more active than the vitamin itself (*12*) and (ii) the increased growth in pigs that is attributed to copper sulfate (*13*). To this list may be added the reactions obtained by adding graded levels of sodium chloride to the diet of crickets. The dose-response curves obtained are extremely atypical for an essential nutrient, but they strikingly resemble the responses obtained in hormoligosis.

Day-old crickets, *Acheta domesticus* (*L*), were placed in glass or plastic specimen jars covered with cheesecloth. Each jar contained a crumpled 7-cm filter paper on the floor for cover; a cotton-stoppered 25-ml erlenmeyer flask with distilled water for drinking; and dry, powdered diet—grass (*14*) with graded amounts of sodium chloride—placed in one corner. The crickets were the offspring of several females and were all fed from the same mix. Sodium chloride solution was mixed into the grass with washed neoprene gloves. More grass was added to give the desired concentration of Na^+ in the food. The jars were placed at random in one tray of an egg incubator at 37°C and relative humidity of 65 percent. The crickets were weighed individually at 30 days, and the average weights were either plotted in milligrams or converted to relative weights (the weights of crickets fed unsupplemented grass was taken as unity).

The response to graded low levels of sodium chloride resulted in multiple stimulation peaks—a growth pattern which resembled that of hormoligosis rather than the smooth response curve expected for an essential nutrient. These data (Fig. 1) are quite similar to those obtained by Richet with different concentrations of salts of heavy metals in lactose fermentation. The two lower curves are based on data obtained in one laboratory; the two upper curves were derived in another laboratory. As the same batch of diet, the same hatch of crickets, and the same time periods were used in the two experiments, the differences are not easily explained. The results at 60 and 100 days were similar to those presented in Fig. 1. The data indicate an instability of the effect of sodium upon the growth of grass-fed crickets.

The experiment was repeated with fresh materials. Average results for 18 crickets (three separate cages of six

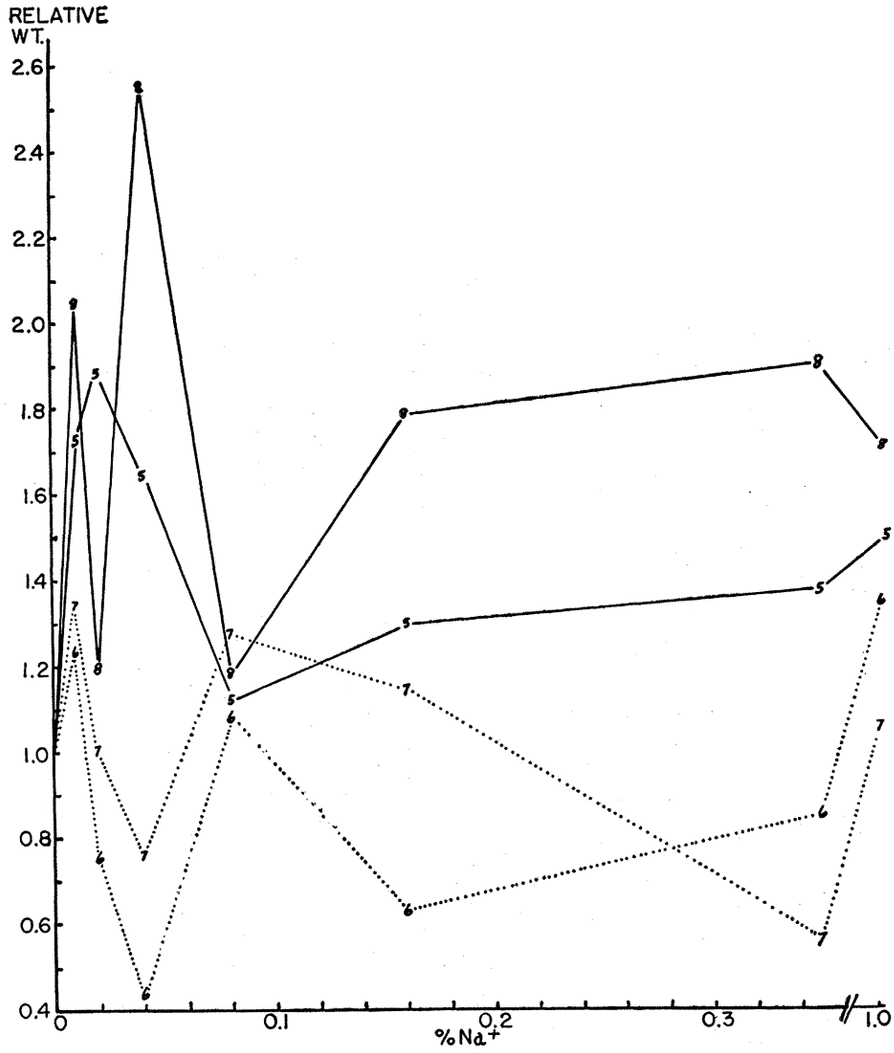

Fig. 1. Response of crickets to sodium chloride. Points 6 and 7 are average values from six crickets reared individually. Points 5 and 8 are average values from 12 crickets reared in two groups of six each.

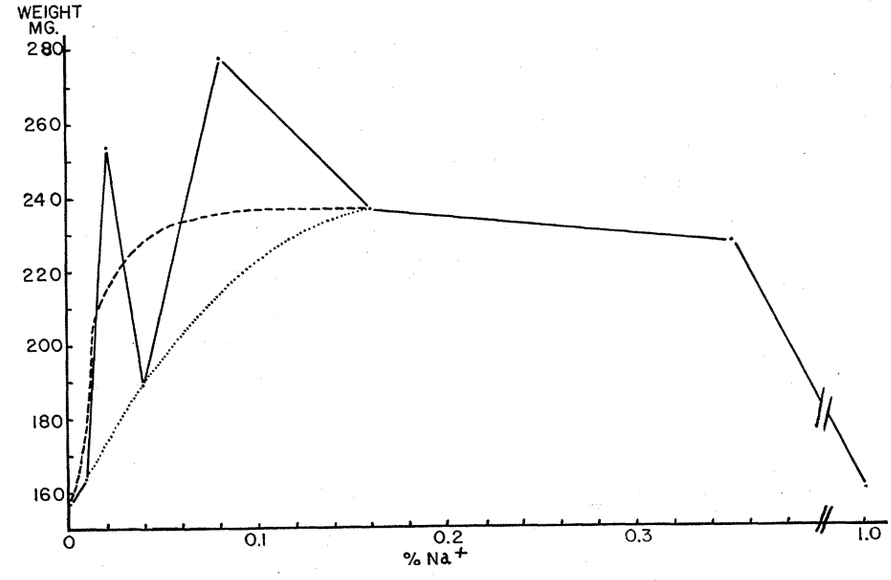

Fig. 2. Response of crickets to sodium chloride.

crickets each) are represented by each point in Fig. 2, to show the actual weight obtained with different amounts of sodium chloride added to the grass. The pattern is very similar to the results obtained previously. All the points above 200 mg are significantly different from the base at the 0.001 level. The weight of crickets fed 0.04 percent Na^+ is not significantly different ($p = 0.04$) from that of crickets fed unsupplemented grass. Crickets fed either 0.02 percent or 0.08 percent Na^+ were significantly heavier ($p = <0.001$) than those fed 0.04 percent Na^+. The amount of sodium in the unsupplemented grass was 0.023 percent in this experiment. The ratios of sodium found by analysis to added sodium are as follows: 0.030:0.01; 0.179:0.10; 0.329:0.35; and 0.882:1.00.

The sodium requirement of the cricket could be presented by the dotted line in Fig. 2. However, this ignores data which do not conform to the classical growth curve. The dashed line represents the median of all points and may approximate the actual requirement of Na^+ as an essential nutrient. But repeated observation of the peaks in the growth response with low levels of sodium chloride suggests that these are actually a part of the biological activity spectrum. The pattern is almost identical with results obtained by Richet (*5*) and could be interpreted as hormoligosis—the stimulation that is caused by a relatively small quantity of an agent which is harmful in large quantities.

An interpretation of hormoligosis by this particular agent, sodium chloride, poses two problems. First, while other agents of hormoligosis are clearly harmful (bactericides, heavy metals, antibiotics, irradiation, and so on), sodium chloride is harmful only in the sense that too much of any agent can be harmful. Most essential nutrients have known toxicities when taken in excess. The more important problem is how the hormetic action of an essential nutrient can be distinguished from its nutritive action. Attention must be given to detailed examination of the complete spectrum of the biological activity of nutrients at different concentrations. Presumably one concentration of a compound may stimulate some systems and depress others.

A possible generalized mechanism for hormoligosis has been presented previously (*9*). In the absence of information on water balance, body composition, food consumption, and food efficiency, a specific mechanism for the data presented cannot be given. This particular effect could be mediated through taste or smell as well as through other physiological mechanisms.

The importance of hormology in nutrition lies in the emphasis it places upon the concept that stimulation of one or more systems by a compound or element does not make that compound or element an essential nutrient (*15*).

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Hemoglobin Types of the Caingang Indians of Brazil

Abstract. We studied the hemoglobin types of 440 individuals (306 unrelated) cytologically and by paper electrophoresis. These included 334 (252 unrelated) putative "full bloods." No abnormal types were found.

A great amount of work has already been done concerning the distribution of the abnormal hemoglobins in several regions of the world (*1, 2*). However, the North and South American Indians and Eskimos have not been studied as intensely as other populations. In regard to South America, the only records to date of electrophoresis studies are the findings of Arends and Layrisse (*3*) of no abnormal hemoglobins in a small sample of 51 Carib Indians, and of Layrisse *et al.* (*4*) of one AS individual among 103 Guayqueri mestizos; both groups were from Venezuela.

The study of the possible occurrence of abnormal hemoglobins among these Indians, however, could be of great anthropological interest. For instance, hemoglobin E appears with relatively high frequencies among Mongoloid populations, but not among the Chinese (*1*). If the occurrence of hemoglobin E could be demonstrated among the Polynesians and other inhabitants of the Pacific, if its absence could be demonstrated in Siberian and North Mongolian populations, and if it occurred among the American Indians, we would have new evidence for Rivet's thesis (*5*) that the American aborigines originated, at least in part, from Pacific peoples. The study of the hemoglobin types of the American Indians could result, also, in the discovery of new forms that would only occur among them. These considerations led us to include this characteristic in a long-term study which is being performed among the Caingang Indians of Rio Grande do Sul, Brazil (*6*).

The populations studied live in four localities in the northern region of the state of Rio Grande do Sul. Blood was collected by venepuncture and placed in sterilized tubes, which were kept in an ice-box until arrival at our laboratory, 3 or 4 days after the collection. In the laboratory the samples were washed three times with saline, hemolyzed by repeated freezing and thawing, and centrifuged at about 1300 *g*. Afterward their concentration was colorimetrically adjusted to about 8 percent with Veronal. The separations were made in a horizontal paper electrophoresis chamber, with Whatman 3 mm filter paper, Veronal buffer, *p*H 8.6, ionic strength 0.05, field strength 4 volt/cm, and duration of 12 hours. Constant voltage was supplied by a Heathkit power supply model PS-3.

Emmel's cytological test (*7*) was performed also, which consisted in placing a drop of blood between a microscope slide and coverslip, and in isolating it from the air with a mixture of paraffin and wax. After 48 hours, the preparation was screened for the presence of sickle cells.

A total of 440 individuals were studied, approximately 100 from each locality; 334 of them were classified as "full bloods," and 106 as mestizos, on the basis of their morphological appearance and, in some cases, by interrogating them or other Indians about possible white or Negro ancestry. Of these, 252 "full bloods" and 54 mestizos were unrelated, as shown by detailed genealogical records. None of them presented any abnormal hemoglobin. In Table 1 our results are compared with those of several other authors. The findings in individuals with no signs of white or Negro admixture are generally negative, with the exception of the reports of Rucknagel (*8*) and Carbonell and Alemán (*9*). Rucknagel, however, believes that the Hb^8 gene found in his sample can be explained by undetectable crosses with Negroes, and the same could be true for the two AS individuals found by Carbonell and Alemán.

In some individuals of our sample

Table 1. Abnormal hemoglobin surveys in North and South American Indians. *Methods.* (A) Paper electrophoresis and other biophysical methods; (B) paper electrophoresis only; (C) paper electrophoresis and cytological (reducing substance: metabisulphite); (D) cytological only; (E) cytological only, (reducing substance: hydrosulphite); (F) cytological only, Scriver and Waugh (*17*) method; (G) paper electrophoresis and cytological, Emmel (*7*) method.

Population	Method	Frequency of individuals with abnormal Hb	No. of individuals tested
Eskimos, Alaska, U.S.A. (*12*)	(A)	0	708
Indians, Alaska, U.S.A. (*12*)	(A)	0	44
Aleuts, U.S.A. (*12*)	(A)	0	200
Indians, Alaska, U.S.A. (*13*)	(B)	0	169
Several tribes, U.S.A. (*8*)	(C)		
Indians		AS(0.75)	133
Mestizos		AS(1.59)	126
Cherokee, North Carolina, U.S.A. (*14*)	(C)		
Indians		0	136
Mestizos		0	398
Lumbee, (Indians and mestizos), North Carolina, U.S.A. (*14*)	(C)	AS(1.73) AC(1.73) AD?(0.08)	1332
Caribs, Venezuela (*2*)	(C)	0	51
Guayqueri mestizos (*3*)	(B)	AS(0.97)	103
Serra de Perijá Indians, Venezuela (*9*)	(D)	AS(1.64)	122
Several tribes from Amapá, Maranhão and Mato Grosso, Brazil (*15*)	(E)	0	1379
Fulniô mestizos, Pernambuco, Brazil (*15*)	(E)	AS(1.81)	166
Guaranis, Nonoai, Rio Grande do Sul, Brazil (*16*)	(F)	0	47
Guarani mestizos, same locality (*16*)	(F)	0	5
Caingangs, same locality (*16*)	(F)	0	27
Caingang mestizos, same locality (*16*)	(F)	0	22
Caingangs, Rio Grande do Sul, Brazil (this report)	(G)	0	334*
Caingang mestizos, Rio Grande do Sul, Brazil (this report)	(G)	0	106†

* Of which 252 are unrelated † Of which 54 are unrelated.