The effect of ethionine on the induction of the increased metabolism of pentobarbital and meprobamate and the increased urinary excretion of vitamin C induced by the pretreatment with phenobarbital or chloretone

Pretreatment	Pentobarbital metabolism (μg/g/2 h)	Meprobamate metabolism (μg/g/2 h)	Total vitamin C excretion (μg/100 g/day)	Ascorbic acid excretion (μg/100 g/day)
–	138 ± 3.3 (6)	145 ± 4.1 (6)	635 ± 48 (16)	481 ± 35 (16)
Phenobarbital	389 ± 5.3 (6)[a]	421 ± 5.4 (6)[a]	1885 ± 204 (8)[a]	1423 ± 118 (8)[a]
Chloretone	242 ± 4.5 (4)[a]	250 ± 4.8 (4)[a]	4641 ± 345 (12)[a]	3838 ± 209 (12)[a]
Ethionine	133 ± 4.0 (4)	141 ± 4.3 (4)	889 ± 78 (8)[c]	743 ± 82 (8)[b]
Ethionine + phenobarbital	148 ± 4.1 (6)	154 ± 3.9 (6)	2282 ± 305 (12)[a]	1731 ± 129 (12)[a]
Ethionine + chloretone	136 ± 3.2 (4)	138 ± 4.3 (4)	5236 ± 491 (12)[a]	4345 ± 269 (12)[a]

Pentobarbital and meprobamate metabolisms are represented by μg of the drugs metabolized by 1 g of liver slices in 2 h. Urinary excretion of ascorbic acid and total vitamin C are represented by μg of the acid or total vitamin C excreted in 24 h per 100 g body weight. The numbers of rats used is shown in the brackets. Probability = [a] $P < 0.001$, [b] $P < 0.01$, [c] $P < 0.05$.

carried out as described previously[6]. Ascorbic acid and total vitamin C were determined by 2,4-dinitrophenylhydrazine method and the 2,6-dichlorophenolindophenol method respectively.

The Table shows that ethionine has a potent inhibitory action on the induction of an increase in the drug metabolism in liver, while, on the contrary, it does not show any inhibitory effect on the increase in urinary excretion of vitamin C.

On the other hand, it was also observed that phenobarbital has a marked action on the increase in the metabolism of drugs, but it has a moderate effect on the increase in the urinary excretion of vitamin C. Chloretone, on the contrary, has a marked effect on the increase in the urinary excretion of vitamin C, but it has little effect on the metabolism of the drugs.

The results indicate that if some analogies between the mechanism of the increase in drug metabolism in liver and the increase in urinary excretion of vitamin C exist, the mechanism of the latter cannot be the same as that of the former.

Further experiments with different doses of ethionine showed that the administration of 150 mg/kg, 75 mg/kg, and 100 mg/kg for three successive days inhibited the chloretone induced increase of the drug metabolism, but even the high doses of 300 mg/kg, 200 mg/kg, and 250 mg/kg failed to inhibit the chloretone-induced increase of urinary excretion of vitamin C.

Riassunto. L'etionina è capace di inibire l'aumentata attività metabolica del fegato sul pentobarbital e sul meprobamato indotta da un pretrattamento con fenobarbital o cloretone. Essa invece non inibisce l'induzione dell'aumento dell'eliminazione urinaria di vitamina C causata da un analoge prettamento con fenobarbital o clorotone. Si conclude che i meccanismi delle due induzioni non possono considerarsi identici.

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The Effect of Temperature Variation on Spontaneous Potential Production from Explants of Brain Tissue in Culture[1]

This is the first report of the quantitative response of spontaneous potentials from explants of brain tissue in culture to variations in environmental temperature. It concerns the separate but simultaneous responses of two similar explants of the superficial part of the posterior-lateral aspect of the telencephalon of 14-day chick embryos to the same variation in temperature. The results provide some information concerning the nature of these spontaneous potentials.

Each explant was procured in the usual way[2,3] and each placed on a separate coverglass between and touching the upper surface of a cellulose sponge and a 36 gauge bare platinum electrode. Each coverglass with its sponge, explant and electrode was placed in a separate Kahn tube which had a 36 gauge platinum wire cemented on its wall to serve as a reference electrode. Sufficient supernatant (balanced salt solution TDL1[2] and 0.25% human serum protein) was added to each Kahn tube to cover the end of the reference electrode and come half-way up the cellulose sponge without touching the explant. A serum stopper containing an air filter was inserted into the upper end of each tube in such a way as to allow the egress of the electrodes. The two explants in their separate but identical tubes were then placed in similar places in a double-walled copper incubator with a water jacket heated by an alcohol lamp. When the walls of the incubator were grounded they made a very satisfactory shield against artefacts from transients and interference. The pair of electrodes from each culture tube was connected to separate but identical amplifier systems and paper strip recorders by shielded cables.

After the explants had been 24 h in culture the temperature in the incubator was altered and observations made on the frequency and magnitude of the potentials produced by each explant per unit of time (30 sec). The temperature was altered as follows (Figures 3, 4, and 5); lowered from the original 39°C to 32°C and then brought back to 35°C, observations being made at every half degree change in temperature. From 35°C the temperature was raised slowly to 47°C with continuous recordings being made on the strip chart recorder from each explant. When all activity had ceased in both explants the tem-

[1] This research was supported by a grant from the Office of Naval Research, U.S. Navy Contract NONR 1598(04).

[2] A. W. B. CUNNINGHAM, M. DOUGHERTY, and B. J. Rylander, Nature *186*, 477 (1960).

[3] A. W. B. CUNNINGHAM, Nature *190*, 816 (1961).

perature was raised to 50°C then lowered to 32°C and finally raised again to 39°C at which level the explants were observed continuously for another 24 h.

Results. At the beginning of the investigation, both explants were producing a continuous series of potentials whose slow increase and decrease in magnitude gave them elongated spindle like forms (Figure 1). This feature is regarded by us as characteristic for explants of the telencephalon. In explant A, groups of three potentials of larger magnitude were superimposed on the more continuous series of smaller magnitude potentials (Figure 2). These groups of three potentials were originally two and a half minutes apart but as the temperature rose, this interval increased to 10, then to 15 min and at about

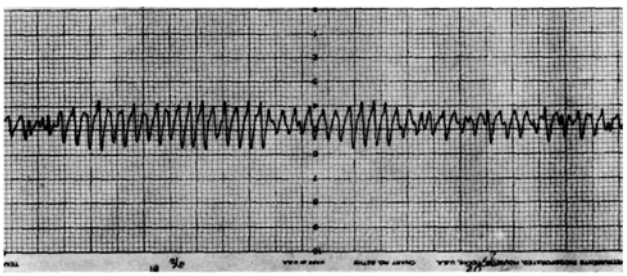

Fig. 1. This shows the elongated spindle form of the paper strip recording of spontaneous potentials from explant B. X-axis, whole length of recording equals 1 min. Y-axis, eight large divisions equals 30 μV.

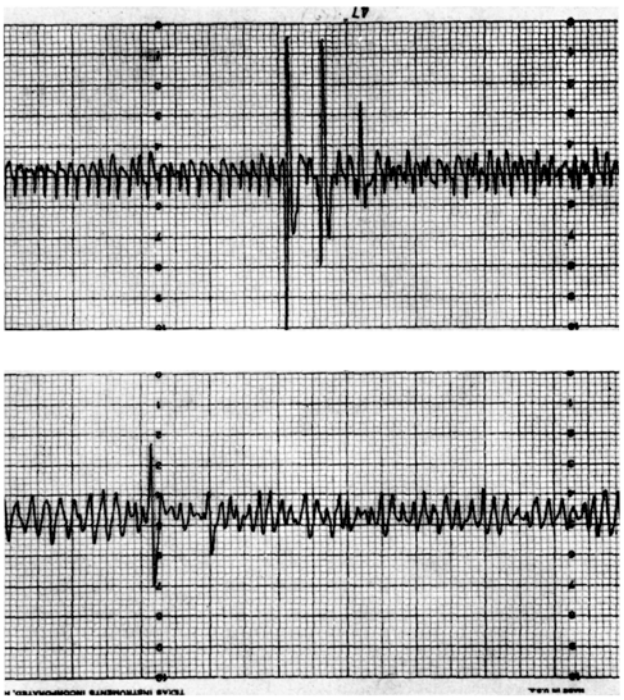

Fig. 2. This shows the paper strip recording of spontaneous potentials from both telencephalic explants (upper trace – explant A, lower trace – explant B). X-axis, two small divisions equals 1 sec. Y-axis, eight large divisions equals 30 μ V.

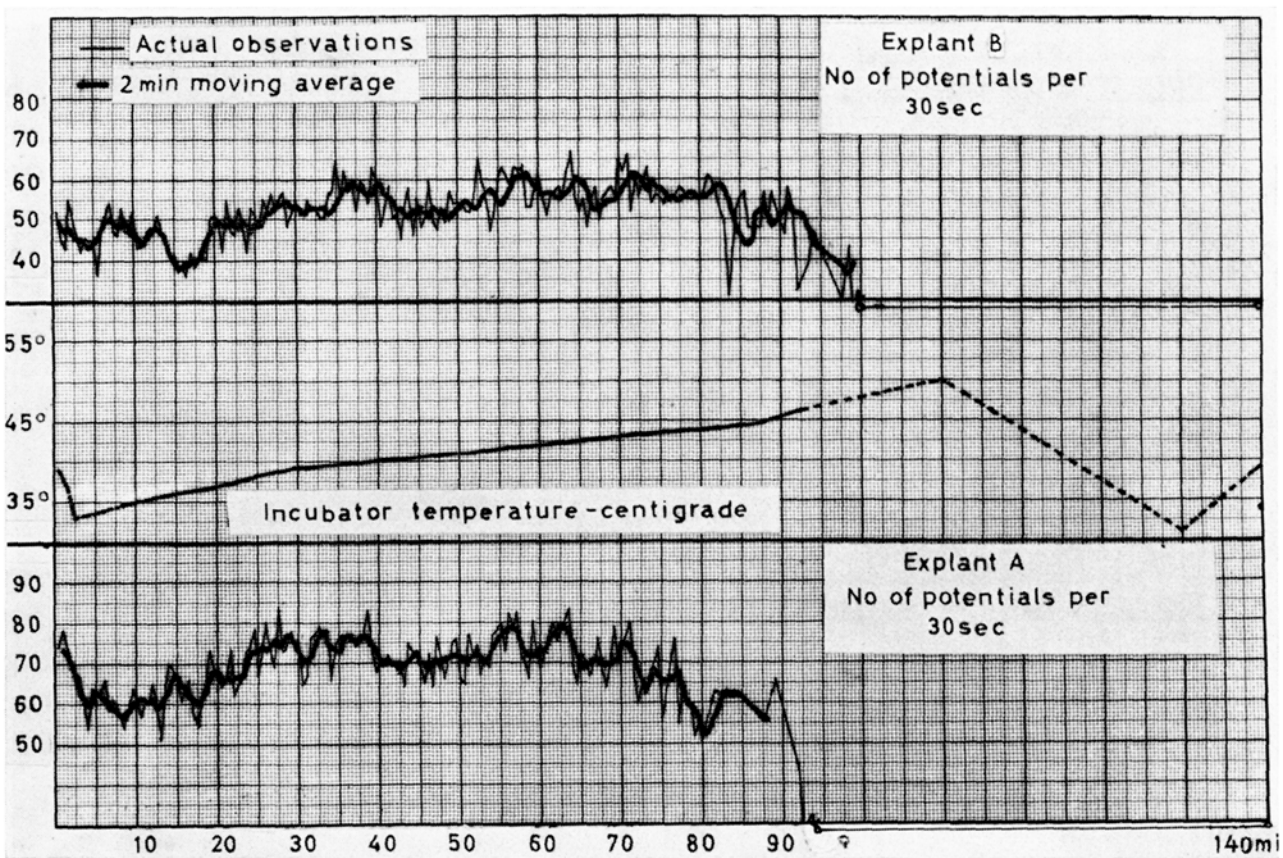

Fig. 3. Shows the change in the frequency of spontaneous potentials from the two explants with change in temperature. X-axis, where there is a broken line in the temperature graph, observations were made on the explants at every 0.5°C change in temperature. Where there is a solid line on the temperature graph, the observations were made continuously. Y-axis, for the graphs explants A and B, number of potentials, for the incubator temperature graph, in °C.

45°C these groups of large amplitude potentials finally vanished. The tall sharp peaks in the 'actual observation' of explant A in Figure 4 are due to these groups of large amplitude potentials. It was considered from our past experience that these groups of large potentials arose from a focus of potential production separate from that causing the more continuous series of potentials and they were not included in the calculation and construction of the curves.

Method of Measurement. The frequency of potentials per 30 sec were obtained by counting the number of changes in potentials which occurred on the strip chart recording for this period of time. We were interested in the quantitative change in the potentials in response to temperature variation and not in the measurement of absolute values for these potentials. We therefore measured the potential per 30 sec period by planimetry of the area under the ink recording of the potentials on the strip chart recording for this period of time. The average potential per spike value was obtained by dividing the potential per 30 sec period by the number of potential spikes for the same 30 sec.

Frequency of potential discharges per 30 sec (Figure 3). The change in the frequency of potential discharges with temperature variation was very similar for the two independent explants. The general trend of change was a fall in frequency with the initial fall in temperature then a slow increase as the temperature rose with a maximum frequency for both explants at about 42°C followed by a decline till all activity ceased at about 47°C. Activity did not return during the subsequent changes in temperature (a rise to 50°C, then a fall to 32° and a final 24 h survey 39°C). When plotted against time, the frequency of potential production in both explants showed a definite cyclic trend. This cyclic trend was very similar in timing and period (3.9 min for explant A and 4.0 min for explant B) in both explants. Explant A responded more rapidly to temperature variation than did Explant B as can be seen; (a) in the response to the initial drop in temperature, (b) in the fact that the cyclic variations in the frequency of spike production occur in explant A slightly before those in explant B, (c) the increase in temperature above 42°C causes cessation of potential production in explant A first. The frequency of spike production increased approximately 1.3 times for a 10°C increase in temperature in explant A and 1.45 times for the same rise in explant B.

Magnitude of potential per 30 sec (Figure 4). The magnitude of potential from both explants drops with the initial fall in temperature and then rises with the increase in temperature to reach a maximum value at about 37.5°C. In spite of a continued rise in temperature the magnitude of potential production in both explants falls slowly till about 47°C after which all activity ceases and does not return with any of the subsequent changes in temperature during the subsequent 24 h. When plotted against time, the magnitude of potentials produced by each explant follow a cyclic course in response to a uniform increase in temperature. The cycles have an average of 4 min for both explants A and B. Again, explant A seemed to respond a little more rapidly to changes in temperature than did explant B. The magnitude of the average potential for explant A at 35°C was 1.54 times that at 45° while that for explant B at 35°C was 1.83 times that at 45°C.

Average potential per spike (Figure 5). The variations in this value with changes in temperature were very similar for both explants. There was a minor reduction with the

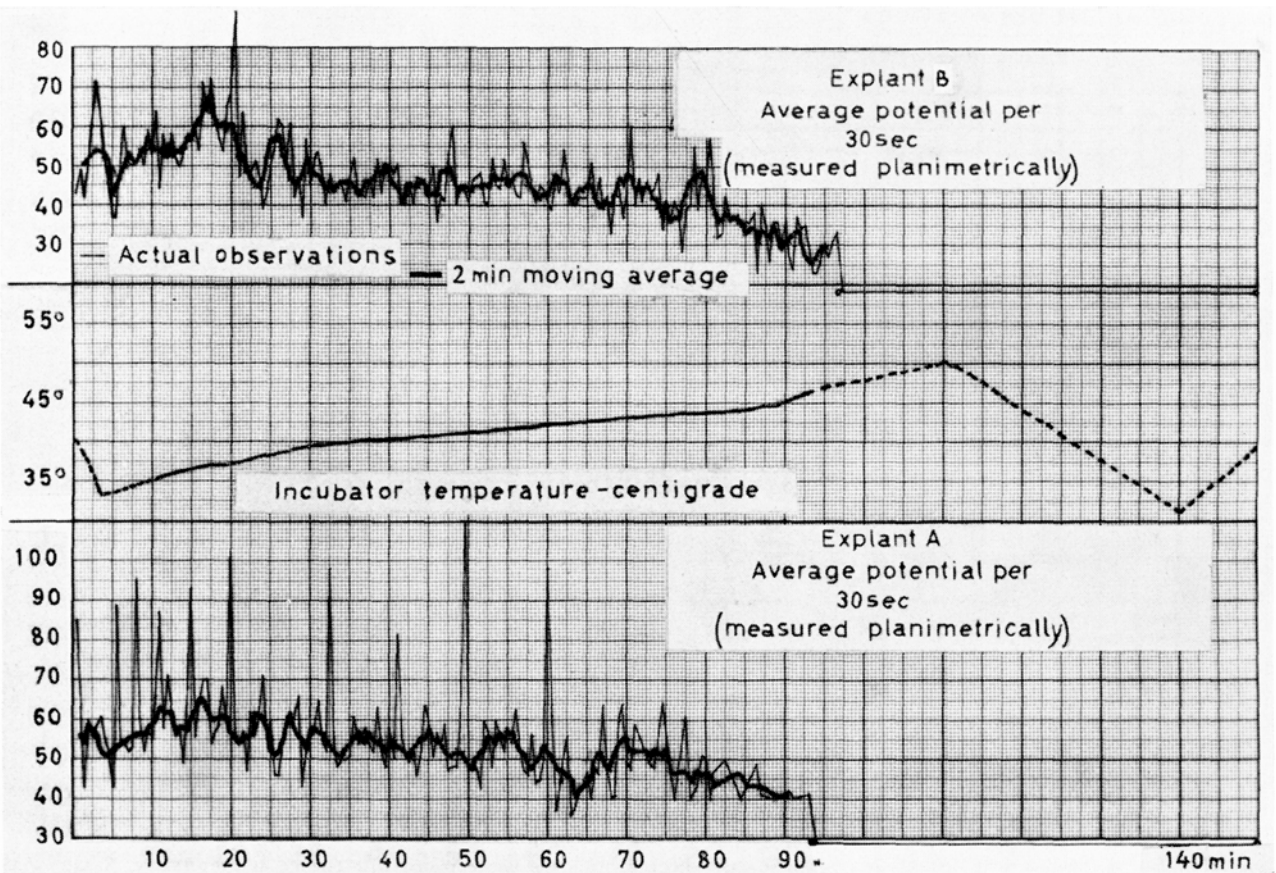

Fig. 4. Shows the change in the magnitude of spontaneous potential per 30 sec for the two explants caused by the temperature variation. X-axis, as for Figure 3. Y-axis, for the graphs for the two explants, the arbitrary planimetric values for the average potential per 30 sec.

initial drop in temperature then a short-lived rise till 37°C, a slow fall till the temperature was between 42.5 and 43.5°C and a terminal rise, followed by a permanent cessation of activity. Explant A shows the effects of the temperature change slightly before explant B, but the values for explant B are much higher than those for explant A until 37.5°C (due to the occurrence of a more definite 'spindle form' in the potentials from this explant during this period). The potential per spike value for explant A at 33°C is 1.7 times the same value at 43°C and the potential per spike value for explant B at 35°C is 2.0 times that for 45°C.

Discussion. Since the observations in this study were made on only two explants, conclusions drawn from them have a limited significance. However, since this is the first time that such a study has been made, it is of interest to explore the observations as thoroughly as possible in a tentative fashion. In general the explants show a reduction in activity with an initial drop in temperature followed by a return to normal when the temperature returns to normal and an initial increase in activity when the temperature is raised above normal followed by a reduction in activity then a cessation of activity when the temperature reaches 47.5°C. Activity did not return in either explant when the temperature was subsequently reduced to and below normal. This type of response strongly suggests that the production of potentials is dependent on a living process. The striking similarity in response on the part of the two independent explants is not likely to have been due to chance. The two responses were not synchronous and the interval between their responses was too long to have resulted from the same transient external electrical interference on the two explants. The delay of the response of explant A with regard to that for explant B may be due to a lesser thickness of explant A allowing more rapid penetration of a temperature change, a greater sensitivity of explant A to temperature change or possibly the active focus in explant A was nearer to the source of heat than the focus in explant B.

The cyclic behavior of both explants is of interest for it is characteristic of living tissue. The cycles are not due to the spindle form of the potentials for the period of the spindles differs in the two explants and is not the same as the period of the cycles.

Since the temperature was constantly changing, it was impossible to get sufficient observations on the activity of the explants at any one temperature to enable us to calculate a significant Q 10 value. The situation was further complicated by the cyclic change in activity which made it necessary in the calculation of Q 10 to use either the average of observations during a whole cycle at the two desired temperatures or to use the value from the same phase of the cycle in each case.

Using another explant from a 12-day chick embryo Telencephalon which had been in culture for 24 h the following Q 10 values were measured for the change in activity between 12 min representative activity at 29° and 12 min representative activity at 39° c/c. The Q 10 value for the number of impulses per unit of time was 2.67. The Q 10 value for the amount of potential produced per unit of time was 1.5 and that for the amount of potential per impulse was 1.79. These Q 10 values are comparable with those previously reported for functional activity of the brain.

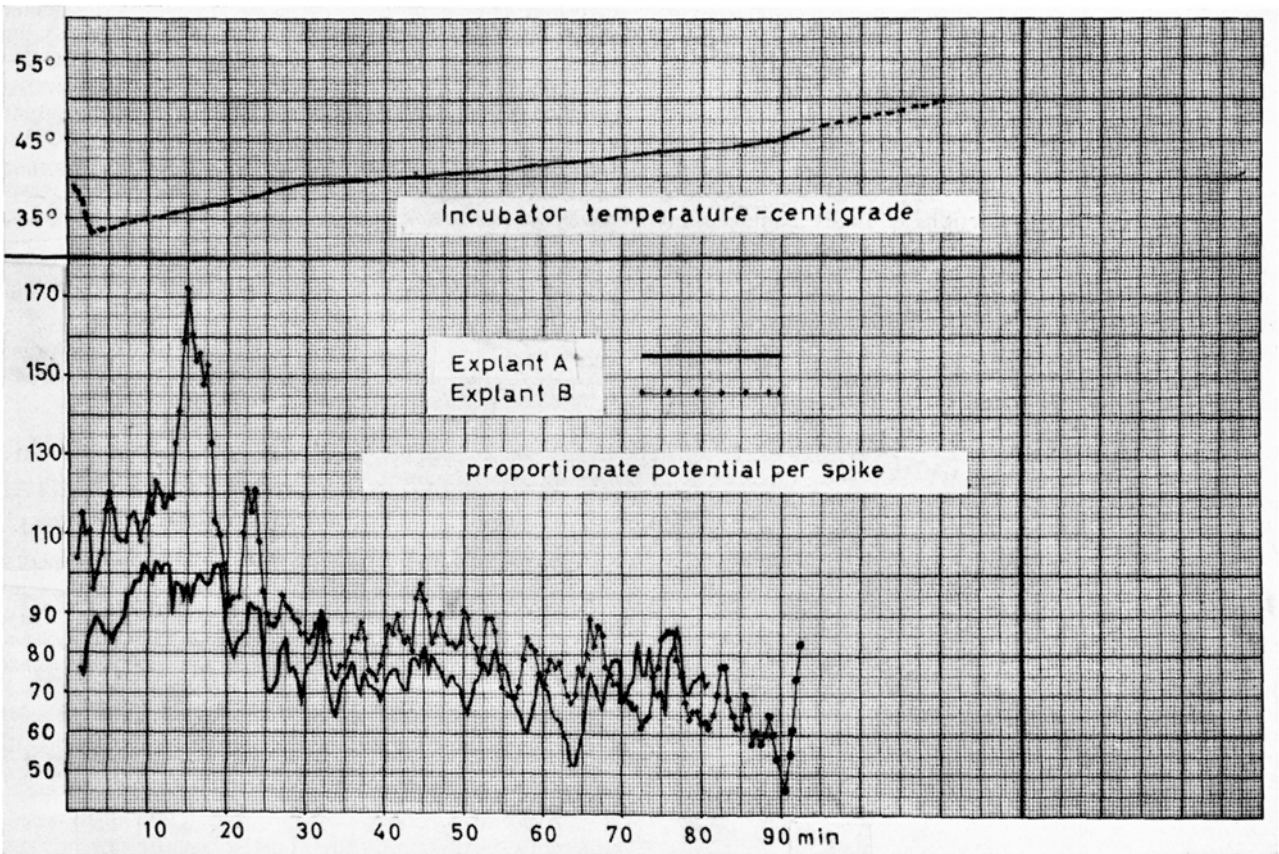

Fig. 5. The change in potential per spike for the two explants resulting from the variation in temperature. X-axis, as for Figure 4. Y-axis, arbitrary units of potential (planimetric values) per spike.

In both explants, the values for the potential per spike rose during the period that the permanent temperature damage was being inflicted. The frequency of the potentials fell more rapidly than their magnitudes for the same period of time. The difference in the general response of these two aspects of the behavior of the spontaneous potentials and in the effect of the final temperature damage suggests that they may depend upon different underlying mechanisms.

As soon as the experiment was over, both explants were fixed in buffered formalin, processed and stained for histological examination. They both showed the presence of histologically healthy examples of the various normal cellular elements of the telencephalon.

Zusammenfassung. Bei zwei verschiedenen Explantaten von Telencephalongewebe 14tägiger Hühnchenembryonen ergab sich als Temperaturabhängigkeit der Aktionspotentiale: Frequenz und Amplitudenabnahme bei sinkender, Zunahme bei steigender Temperatur bis zwischen 37 und 42,5°C. Weitere Temperatursteigerung bringt die Potentiale nach stetiger Verkleinerung bei 47°C schliesslich zum Verschwinden.

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The Antimicrobial Activity of some Insect Extracts Possessing Juvenile Hormone Activity[1]

The juvenile hormone of insects acts to promote larval syntheses and thus deters adult differentiation. During a general study of the distribution of this molecule, substances possessing juvenile hormone activity have been extracted from vertebrate material (GILBERT and SCHNEIDERMAN[2]; WILLIAMS, MOORHEAD, and PULIS[3] from invertebrates other than insects (SCHNEIDERMAN and GILBERT[4]) and from plants and microorganisms (SCHNEIDERMAN, GILBERT and WEINSTEIN[5]). As part of a broad program to investigate the physiological significance of the ubiquity of this biologically active substance, juvenile hormone extracts were tested on bacteria. The data to be presented show that some of these extracts possess antimicrobial activity against a variety of microorganisms and that such activity is associated with the fatty acid component.

Methods. Unless stated otherwise, ether extracts of adult giant silkmoth abdomens, (*H. cecropia*, *S. cynthia*, and *R. orizaba*) were used. The isolation procedure consisted of repeated extractions of finely homogenized abdomens with peroxide-free ether, followed by washing and evaporating to dryness *in vacuo*. The residual oil contains nearly all the ether-soluble material of the abdomen and is rich in juvenile hormone activity (WILLIAMS[6]).

Due to the lipid nature of the test extracts it was not possible to assay the extracts by tube culture or by impregnation of paper discs. A more suitable assay was developed by placing known quantities of extract into holes cut into seeded agar with a sterile No. 4 cork borer. Plates were seeded by adding 0.3 ml of 24 h trypticase soy broth culture of the test microorganism to trypticase soy or nutrient agar. The assay dishes were incubated at 30° C or 37° C depending on the optimum temperature for growth of the microorganism and read at 24 h by measuring the diameter of the inhibition zone in millimeters. Diameter of the cork borer and agar cut-out was 9 mm. Results are expressed as the radius of inhibition or one-half of the total zone diameter minus the diameter of the agar cut out.

Results. Table I reveals the extent of inhibition of microbial growth by extracts of male *cecropia* and male *crizaba* on a wide variety of microorganisms. Royal jelly containing the known antimicrobial agent 10-hydroxy-δ-2-decenoic acid (BLUM, NOVAK and TABER[7]; MCCLESKEY and MELAMPY[8]) was also assayed. While the antimicrobial

[1] Supported by Grants G-7597 from NSF and A-2818 from U.S.P.H.S.

[2] L. I. GILBERT and H. A. SCHNEIDERMAN, Science *128*, 844 (1958).

[3] C. M. WILLIAMS, L. V. MOORHEAD, and J. F. PULIS, Nature *183*, 405 (1959).

[4] H. A. SCHNEIDERMAN and L. I. GILBERT, Biol. Bull. *115*, 530 (1958).

[5] H. A. SCHNEIDERMAN, L. I. GILBERT, and M. WEINSTEIN, Nature *188*, 1041 (1960).

[6] C. M. WILLIAMS, Nature *178*, 212 (1956).

[7] M. S. BLUM, A. F. NOVAK, and S. TABER, Science *130*, 452 (1959).

[8] C. S. MCCLESKEY and R. M. MELAMPY, J. Econ. Entomol. *32*, 581 (1939).

Tab. I. Inhibition of microbial growth by insect extracts

Microorganism	Male *H. cecropia*				Extract Male *R. orizaba* Radius of inhibition (MM)				Royal jelly			
	1	2	3	4[a]	1	2	3	4	1	2	3	4
M. luteus	1.5	2.0	3.0	3.0	0	0	0.5	1.5	7.5	10.5	10.5	13.0
S. aureus	0	0	0	0	0	0	2.0	3.0	0	0	0	0
S. lutea	0	0.5	1.0	1.0	0	0	0	0	3.5	5.5	8.0	8.5
M. smegmatis	2.0	2.5	2.5	3.0	0	0	0	0	3.5	6.5	9.0	11.5
B. megaterium	0	0.5	0.5	1.0	0	0	0	0.5	3.5	5.5	8.5	9.5
B. subtilis	0.5	1.0	1.5	2.5	Not run				4.0	5.0	6.0	6.5
A. fecalis	0	0	0	0	2.5	3.0	4.5	5.5	0	0.5	1.0	1.5
A. aerogenes	0	0	0	0	1.5	4.0	4.5	5.5	0	0	1.0	4.0
E. coli B	0	0	0	0	1.5	4.5	6.5	7.5	0	1.0	1.5	2.5
S. marcesens	0	0	0	0	0	0	1.5	2.0	0	1.0	1.5	2.0
P. vulgaris	0.5	1.0	1.0	1.5	6.0	7.5	8.5	10.5	1.5	3.5	4.5	7.5
S. ellipsoideus	0	0	0	0	0	0	1.5	2.0	0	0	0	0

[a] 1, 0.03 ml; 2, 0.06 ml; 3, 0.09 ml; 4, 0.15 ml.