Tryptophane Synthesis in Insects

Hideo Kikkawa

Sericultural Experiment Station, Taketoyo, Aichi, Japan

According to recent works on *Neurospora crassa*, the metabolism of tryptophane in this mold is represented as follows: Anthranilic acid → indole + serine → tryptophane → kynurenine → hydroxykynurenine → hydroxyanthranilic acid → nicotinic acid (*1, 6, 7*). Furthermore, my genetic and biochemical studies on silkworms show that riboflavin, pterins, and uric acid have a close bearing on tryptophane metabolism from tryptophane or kynurenine as in the case of eye pigments in insects (*4, 5*). It is therefore of great interest to examine in detail the metabolism of tryptophane in insects. In order to find out whether or not insects are able to utilize anthranilic acid or indole as a precursor of tryptophane, the following experiment was performed.

TABLE 1

Substance injected	Kind of pupa			
	Normal type (+ chromogen)		White-1 type (Kynurenine)	
	♀*	♂*	♀*	♂*
1. 0.5% Anthranilic acid ..	98.0	94.9	138.2†	132.2†
2. 0.5% Anthranilic acid + 0.5% L-serine	95.2	92.3	131.0†	129.9†
3. 0.5% Indole	102.7	105.6	99.3	90.4
4. 0.5% Indole + 0.5% L-serine	103.1	93.8	94.3	93.8
5. 0.5% L-Tryptophane	142.9	131.6	136.4	126.1
6. H_2O (Control)	100.0	100.0	100.0	100.0

* Values represent index numbers based on a control value of 100.

† The increase of index number is not due to the increase of kynurenine itself.

At the pupal stage in silkworms food intake and excretion stop completely, although gas metabolism continues. Taking advantage of this fact, we selected 120 pupae for investigation, immediately after pupation. These were divided into six groups of equal number, each group consisting of 10 males and 10 females. Individual pupae in each of the six groups received by injection 0.05 ml of a certain substance which was to be tested as a possible tryptophane precursor. Substances tested were: (1) 0.5% anthranilic acid, (2) 0.5% anthranilic acid + 0.5% serine, (3) 0.5% indole, (4) 0.5% indole + 0.5% serine, (5) 0.5% tryptophane, and (6) distilled water as control. Injected pupae were kept in a room where the temperature was maintained at 23°–25° C for 96 hr. Five pupae of the same sex were picked from each group, and they were separately ground with a certain volume of either 10% trichloroacetic acid or 80% ethanol. For each extraction, variation of the injected substance was examined.

If anthranilic acid or indole is utilized as a precursor of tryptophane in insects, the amount of + chromogen (it is the same with cn+ substance and is assumed to be hydroxykynurenine, according to a personal communication from Prof. Beadle) should increase in pupae of normal type as compared with the control (H_2O injection group). This is the case because in the normal pupae the greater part of free tryptophane is converted into + chromogen. Similarly, in pupae of white-1 mutant type, the amount of kynurenine should increase, because in these pupae the greater part of free tryptophane is converted into kynurenine (*2, 3*). The amount of + chromogen was measured with Ehrlich's diazo reaction method, and the amount of kynurenine with Otani-Honda's method (*2*).

The results of the experiment, as given in Table 1, show that, in both normal males and females, no marked increase of + chromogen was found except in the group in which tryptophane was injected. However, in cases where the white-1 mutant pupae were used, an increase of kynurenine was recognized in the groups which received (1) anthranilic acid, (2) anthranilic acid + serine, and (5) tryptophane. The curious result that the injection of (1) or (2) gave rise to an increase of kynurenine has been clarified by the subsequent experiments. It was ascertained that anthranilic acid injected in either the normal or the white-1 mutant pupae was converted into some unknown substance different from kynurenine, and this substance showed a reaction similar to that of kynurenine with Otani-Honda's method. No increase of indole was recognized in the extraction of groups 1 and 2, in either the normal or the white-1 pupae.

Furthermore, evidence that indole is not a precursor of tryptophane has been shown by the fact that injected indole was detected as such. Addition of serine had no bearing on tryptophane synthesis in insects. From these results, there is left little doubt that anthranilic acid or indole is not utilized as a precursor of tryptophane in insects, at least at the pupation period of the silkworm. It has been found that these unnecessary substances were excreted with urine at last.

Thus it is assumed that the origin of tryptophane in insects is different from that in molds. It may be assumed that the greater part of tryptophane is derived from food, i.e., mulberry leaves. It is possible, however, that indole derivatives, but not indole itself, are converted into tryptophane in insects. The following indole derivatives were tested: skatole, indole acetic acid, indole propionic acid, and indole lactic acid. Among these substances, only indole lactic acid gave a positive result. Similar results were obtained in *Drosophila*. In this case, the substance in question was added to a culture medium, and the increase of + chromogen or kynurenine in pupae was tested by the method described in my previous paper (*3*).

From these experimental results, it may be concluded that the mechanism of tryptophane synthesis in insects is analogous to that of mammals, and not to that of molds. If we assume that the genes control the enzyme functions or chemical reactions, the results mentioned would indicate that the gene complex in insects is not the same as that of molds with respect to tryptophane

synthesis. Presumably, the genes for converting anthranilic acid or indole into tryptophane have become inactive or have been lost in the course of evolution of insects, as in mammals.

References

1. BEADLE, G. W., MITCHELL, H. K., and NYC, J. F. *Proc. nat. Acad. Sci.*, 1947, **33**, 155.
2. KIKKAWA, H. *Genetics*, 1941, **26**, 587.
3. ———. *Sanshi Shikenjo Hokoku* (in Japanese), 1943, **11**, 311.
4. ———. *Medicine and Biology* (in Japanese), 1944, **5**, 242.
5. ———. *J. Jap. Chem.* (in Japanese), 1949, **3**, 36.
6. MITCHELL, H. K. and NYC, J. F. *Proc. nat. Acad. Sci.*, 1948, **34**, 1.
7. TATUM, E. L. and BONNER, D. *Proc. nat. Acad. Sci.*, 1944, **30**, 30.

An Instrument for Use in Measuring Electrical Resistance of the Skin[1]

Frederick G. Whelan

Psychobiological Laboratory, Phipps Psychiatric Clinic, Johns Hopkins Medical School, Baltimore

For many years, a dermometer designed by Levine (*1*) has been in use in this laboratory to measure the electrical resistance of the skin in a variety of neurological, physiological, and psychological studies. It proved to be very useful in studies on normal and hypnotic sleep, peripheral nerve injuries and spinal cord sections, and in detecting physiological differences between different types of neuroses and psychoses (*3, 4*). Although for most purposes it gave satisfactory service, it has proved over the course of time to have several shortcomings. It does not have a sufficiently wide range, since it gives accurate readings only up to 4½ million ohms and skin resistance may greatly exceed this value in a number of conditions. It is too fragile and delicate to be moved from place to place without danger of damage. And owing to its sensitive construction, it is too expensive for general use.

To overcome these drawbacks, a new instrument (Fig. 1) has been designed that has all of the advantages of the Levine machine, but none of its disadvantages, and in addition has several new features. At the same current used in the Levine instrument (2 μa) it has a range of from 1,000 ohms to 45,000,000 ohms. Within this range it has an accuracy of about 5%. This degree of accuracy suffices for most skin-resistance studies. The new instrument is much more rugged, is smaller (outside dimensions 7 in. × 12 in. × 3 in.), and more readily portable, weighing 9¼ lb as compared to 20 lb.

Fig. 2 gives the wiring plan. The instrument consists essentially of a voltage source (9- and 90-v batteries), a means for varying the voltage applied to the unknown resistance (between any two of the posts marked 1, 2, 3, and 4), a microammeter (A) for measuring the current, and a voltmeter (V) calibrated directly in ohms at 2 μa.

For resistances up to 4.5 megohms the voltage source is a pair of small 4½-v batteries connected in series. When the power switch (S1) is turned on, this voltage is connected across a series combination of two rheostats. One of these, 500 ohms (P1), is used as a fine adjustment, and the other, 15,000 ohms (P2), is used as a coarse adjustment of the voltage applied to the unknown resistance, which is connected between the two variable taps on the rheostats.

The microammeter (A) has a range of 10 μa on either side of zero. Since in measuring the electrical skin resistance a small current sometimes flows before any external voltage is applied, a zero-centered meter allows this resting current to be measured and compensated for regardless of its direction. The microammeter is automatically short-circuited when the power switch (S1) is turned to the "Off" position.

The voltmeter (V) is a 0–1-ma d-c meter having a resistance of about 100 ohms. A three-position lever switch (S4) is used as a range switch connecting the proper resistances (R1 or R2) in series with the meter to give ranges of 0–45,000 ohms (R), 0–450,000 ohms (R × 10), and 0–4.5 megohms (R × 100).

There are four binding posts (1, 2, 3, and 4 in Figs. 1 and 2) for connecting electrodes to the palm and back of each hand (or to any other four locations) and a jack (J) for plugging in the ear clip and roller electrode combination ordinarily used with the neurodermometer for mapping skin-resistance patterns (*2*). A two-pole, six-position rotary switch (S6) connects the jack or various combinations of the binding posts to the measuring circuit. A double-pole, double-throw toggle switch (S5) is provided in order to reverse the polarity of the electrodes and of the jack. The reversing switch and jack are features not included in the Levine instrument.

In order to measure resistances between 4.5 and 45 megohms, two 45-v batteries connected in series are provided. A double-pole, double-throw switch (S3) connects this 90-v battery in place of the 9-v battery, and at the same time connects the necessary resistance (R3) in the voltmeter circuit to enable this high voltage to be read. The range switch (S4) is disconnected when the 90-v battery is used.

If it is desired to measure resistances below 1,000 ohms, a higher measuring current can be used. For example, by using a current of 10 μa, resistances as low as 200 ohms can be measured. Although the electrical skin resistance as measured at 10 μa is not the same as would have been determined if the measurements were made at 2 μa, nevertheless it does give a numerical value which is a good approximation of the 2-μa value. Similarly, for measuring resistances above 45 megohms, lower measuring currents can be used. A double-pole, double-throw switch (S2) disconnects the internal microammeter, connecting in its stead two binding posts on the panel to which an

[1] Carried out under a contract between the Medical Division, Chemical Corps, U. S. Army, and The Johns Hopkins University. Under the terms of this contract the Chemical Corps neither restricts nor is responsible for the opinions or conclusions of the author