boreus. In slugs, too, the metacercariae occur within the pericardial cavity. Only two species of land snails thus far examined (*A. alternata* and *P. fraudulenta*) harbor this species to the exclusion of all other brachylaemids. The number of metacercariae found within the pericardial cavity varies from one to many. Particularly in *A. alternata*, the number per snail is frequently above 50, and one snail contained 83 fully developed metacercariae. In general, metacerariae within the pericardial cavity may be divided into three recognizable age groups. Those fully developed are ovoidal, with well-established genital fundaments and exhibiting little or no movement. Very young metacercariae are characterized by the presence of a rudimentary tail, whereas intermediate forms are more elongate and tailless and show a considerably greater motility. All specimens differ from other members of the family found in snails in this area by the lack of ciliated excretory tubules. All three age groups may be present within the pericardial cavity of the same snail, indicating that immunity to the cercariae is not established following infection.

Observation showed that entrance of cercariae to the pericardial cavity occurs via the respiratory aperture, From this point, they are assumed to migrate through the excretory duct to the kidney and thence, by way of the renopericardial connection, to the heart chamber itself. Growth of metacercariae is extremely slow; feeding experiments in progress show that at 18 weeks the metacercariae have not yet reached full size.

When fully developed metacercariae were fed to laboratory-reared *Peromyscus*, adults of varying ages were secured from the cecum of the host. Attachment to the cecal walls and ingestion of blood commence within a very few hours after feeding. Adult flukes have been maintained in *Peromyscus* for as long as 100 days. Egg production commences on the 8th or 9th day, while fully embryonated eggs appear in the feces about the 20th day. All attempts at securing discharge of eggs from living worms have been unsuccessful.

Published data dealing with members of this family have shown a conspicuous lack of information concerning the miracidial stage, primarily because of difficulties encountered in freeing the miracidia from the egg shells. Miller (*2*) concluded that the miracidial stage is nonexistent for this form. Hatching of the miracidium of *P. laruei* has been observed by me, and work is now in progress in an attempt to determine its morphology. Movement of the miracidia within the eggs can be seen after the crushed adults have been placed in saline; but emergence was noted only after the adults had been kept under refrigeration several days in normal saline and then transferred to tap water, crushed, and the eggs removed.

Eggs containing fully developed miracidia were fed to laboratory-reared specimens of *A. alternata* and various species of *Polygyra*. Branching sporocysts containing immature cercariae were found only in *A. alternata* after several weeks, while mature cercariae emerged between 3 and 7 months after exposure, depending upon temperature conditions. It is noteworthy that, although snails infected with the metacercarial stage are abundant in nature, averaging from 85 to 100% infection in localities near Ann Arbor where this parasite is found, the number of snails harboring sporocysts and cercariae is exceedingly small, since only 15 individuals of more than 3,200 examined to date by techniques involving the crushing of the snail have shown these stages. Moreover, fully developed cercariae emerged from only 5 of these 15. During the examination of a great variety of terrestrial snail hosts, sporocysts of other species of Brachylaemidae have been found, but those of *P. laruei* were found only in *A. alternata*, indicating specificity of this species of snail to infestation with the sporocyst of *P. laruei*.

A detailed account of this life cycle with complete descriptions of all stages involved is in preparation and will appear elsewhere.

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Distribution of Free Amino Acids in Mouse Epidermis in Various Phases of Growth as Determined by Paper Partition Chromatography

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This is part of a series of experiments the purpose of which is to characterize the nitrogen metabolism of mouse epidermis in various phases of growth.

In the experiments to be reported a survey was made of the free amino acids found in alcoholic extracts of whole epidermis obtained from newborn mice (0–4 and 7 days of age), from normal adult mice, and from mice receiving 3, 6, or 12 applications of methylcholanthrene in benzene or 3 applications of benzene alone. A transplantable squamous cell carcinoma originally derived from a carcinoma produced on the skin of a mouse by the application of the carcinogen was also studied. The method of treatment of the animals and preparation of the tissues has been described (*6*). The tissues were dried *in vacuo* over P_2O_5, ground, and redried to constant weight. Samples (200 mg) of the tissues studied were stirred thoroughly with 1 ml of 76% alcohol at room temperature, and determinations by the two-dimensional chromatographic method (*1, 2, 4*) were made on 75 μl of the untreated extract and on similar aliquots after oxidation with H_2O_2 and after hydrolysis with HCl. The amino

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acids were identified by their relative positions on the paper square and by comparison with a reference chro-

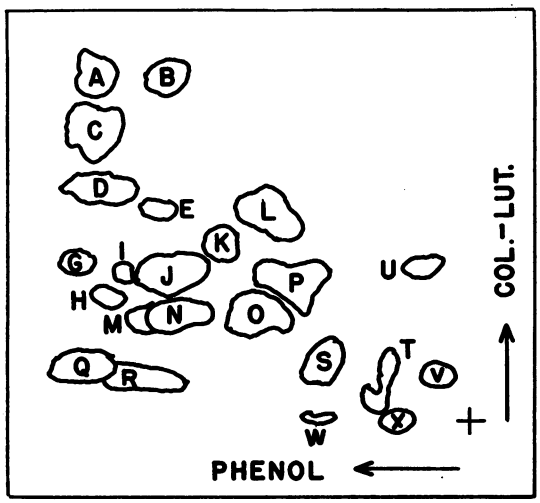

FIG. 1. Diagram of constituents of chromatograms made from epidermal and tumor extracts: A, phenylalanine; B, tyrosine; C, leucine; D, valine; E, methionine sulfone; G, proline; H, histidine; I, hydroxyproline; J, alanine; K, threonine; L, taurine; M, β-alanine or citrulline; N, glutamine or serylglycylglycine; O, glycine; P, serine; Q, arginine; R, lysine; S, glutamic acid; T, aspartic acid; U, cysteic acid; V, "oxidized" glutathione; W, "underglutamic acid," unidentified; X, glutathione.

matogram prepared by Dent (*3*). A diagram showing the positions of the ninhydrin-reactive substances found in the samples is shown in Fig. 1. Spot X was given by

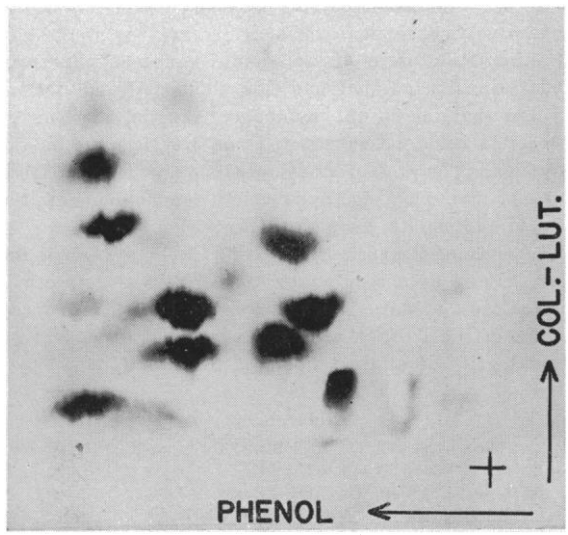

FIG. 2. Peroxide-treated extract of epidermis after 6 paintings with methylcholanthrene in benzene.

glutathione either in pure solution or on addition to a casein digest. On treatment of either solution by H_2O_2, this spot was shifted to position V. It is interesting that, of these two spots, only V was observed in *untreated* extracts of all the tissues examined with the exception of the tumors, in which case only spot X was found.

In a previous investigation (*5*) it was found that the amino acid pattern of whole tumor tissue was significantly different from that of normal epidermis, but similar in most respects to that of the nonmalignant hyperplastic epidermis produced by the application of methylcholanthrene. The present study shows that the carcinoma can be sharply differentiated from the latter tissue on the basis of the distribution of free amino acids in the alcoholic extracts (Figs. 2 and 3). The tissue made hyperplastic by the application of the carcinogen and the epidermis of newborn mice had greater over-all

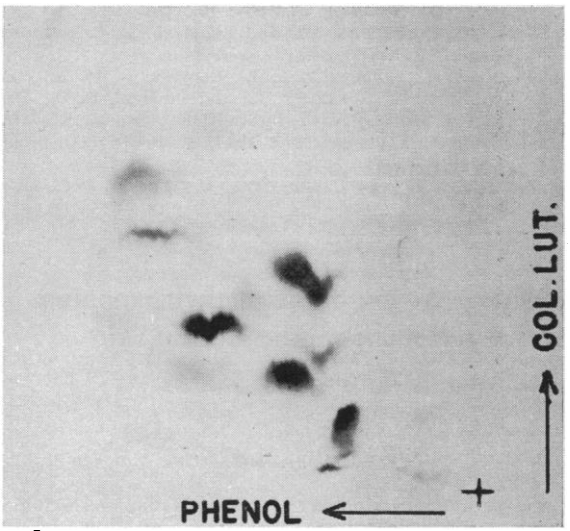

FIG. 3. Extract of squamous cell carcinomata.

concentrations of the detectable constituents than the normal adult epidermis, while the tumors showed a striking decrease. Only the "underglutamic acid" and cystine spots (the latter not appearing on this photograph) in the tumors had a greater intensity than in the other samples. The over-all decrease in free amino acids found in the tumors is even more impressive when it is recalled that a given fresh weight of this tumor contains only approximately one-half of the dry weight found in the same fresh weight of normal or hyperplastic epidermis (*6*). There was a retardation of the movement of the amino acids in the tumor extracts in the phenol direction which disappeared after acid hydrolysis. This phenomenon, which is often associated with the presence of constituents of high molecular weight, such as polypeptides, is under further investigation. The application of pure benzene produced marked increases in proline and lysine contents, a decrease in the taurine level, and little or no change in the other constituents.

The epidermis of newborn mice and hyperplastic epidermis, both of which tissues have a more rapid rate of growth than normal adult epidermis, had greater concentrations of free amino acids than the latter tissue,

while the rapidly growing carcinoma had a much lower content than normal. This suggests that the growth in the nonmalignant epidermis may be associated with the ability of the cells to increase the intracellular concentrations of the amino acids necessary for protein synthesis, whereas in the malignant tumor the mechanisms for protein synthesis are much more efficient and can operate at a greatly accelerated rate, even in the presence of smaller concentrations of amino acids.

Several aspects of this work are being studied further.

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Blocking Action of Tetraethylammonium on Axon Reflexes in the Human Skin

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Tetraethylammonium (*4*), which is now enjoying a wide trial as a diagnostic and therapeutic agent, is believed to exert its effects by the specific blockade of autonomic ganglia (*1, 2*). It is of interest, therefore, that in connection with some studies of the autonomic pharmacology of the skin we have obtained evidence that tetraethylammonium may exert an action on peripheral nerve fibers.

In most individuals the intracutaneous injection of acetylcholine in appropriate concentrations induces pilomotion and sweating, which occur very promptly after injection of the drug and extend for a considerable distance from the site of injection. These responses have been shown to be due to axon reflexes dependent on the integrity of the postganglionic sympathetic fibers (*5*).

By previously infiltrating the skin with tetraethylammonium we have been able to inhibit the axon reflexes of pilomotion and sweating induced by acetylcholine.

Pilomotion was elicited by the intracutaneous injection of 0.1 ml of acetylcholine hydrobromide, 1: 25,000; axon reflex sweating, by the injection of 0.1 ml of acetylcholine hydrobromide, 1: 500. Sweating was demonstrated by the ferric chloride–tannic acid method of Silverman and Powell (*6*). Axon sweating must be distinguished from the local response, which is confined to the area of the wheal, and from the sweating which follows the lymphatic diffusion of acetylcholine. Areas of the volar aspects of both forearms were selected for each test. The control area was prepared by the intracutaneous injection of 0.1 ml of physiological saline. The corresponding contralateral area was prepared by the intracutaneous injection of 0.1 ml of tetraethylammonium chloride, 1: 100.

It should be noted that tetraethylammonium itself, in the range of 1:10 to 1: 100,000, does not induce pilomotion or sweating.

In 15 experiments on 7 subjects in no instance did pilomotion occur when acetylcholine was injected into the spot where tetraethylammonium had been injected 1–2 min previously. Injection of acetylcholine into the control area treated with saline in every test produced typical pilomotion over an area of 2–3 cm in diameter surrounding the injection site and lasting for 45–90 sec. In 12 experiments on 8 subjects axon reflex sweating was either completely inhibited or markedly suppressed when acetylcholine was injected into the spot where tetraethylammonium had been injected 1–2 min previously. The local sweat response was not impaired. Injection of acetylcholine into the control area treated with saline in every instance produced typical axon sweating over an area of 2–4 cm in diameter.

Since these axon reflexes of the skin depend on the integrity of the postganglionic sympathetic axon and since they are blocked by the presence of tetraethylammonium locally in the skin, it is presumed that the blockade occurs along the course of the efferent sympathetic fibers in the skin.

The only other theoretically possible site of the stimulating action of acetylcholine and the blocking action of tetraethylammonium is the neuro-effector junction. In any case, there are no ganglion cells in the skin, although these axon reflexes occurring over the peripheral neural apparatus behave pharmacologically as though the site of stimulation were an autonomic ganglion. Rothman and Coon (*5*) pointed out this similarity and demonstrated that, as in the autonomic ganglia, nicotine or α-lobeline could be substituted for acetylcholine as the stimulus. The present study reveals a further similarity, namely, the blocking by tetraethylammonium of the nicotinic stimulating action of acetylcholine.

Evidence of the specificity of this blocking effect is the fact that we have been unable to inhibit with tetraethylammonium the flare which surrounds histamine-induced wheals of the skin, an axon reflex which is dependent on the integrity of afferent fibers of the skin (*3*).

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