teins can be achieved. This opens up the possibility of using the technique for preparative purposes, since that portion of the paper strip containing a particular fraction can be cut out and the fraction eluted by customary methods.

The fact that convection currents are of no great consequence in ionography will permit liquids other than water to be used. When electrophoretic measurements in water are made at any temperature other than in the neighborhood of 4° C, the point of maximum density, convection currents offer a serious difficulty. As very few nonaqueous liquids or liquid mixtures exhibit points of maximum density, the study of electrophoresis in the past has been restricted largely to water solutions or suspensions. In the technique described here, the elimination of convection currents means that electrophoretic studies can be extended to many organic liquids and to solutions of water with other liquids; this possibility has important implications in biochemical work, where many materials of great interest are soluble in water to only a very limited extent.

Experiments are contemplated—using sheets of filter paper instead of paper ribbon—in which the movement of charged particles or ions would be influenced not only by an electrical field but by a superimposed magnetic field as well. In effect, this system would be equivalent to a mass spectrograph applicable to charged particles in solution.

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Penetration of Benzpyrene into the Stomach Wall of Mouse

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Under what conditions and by which routes the carcinogenic hydrocarbons enter the living organism in general, and the individual cells expressly, are still almost unexplained questions.

Seeking an answer, we, among others, have painted the skin of newborn mice with carcinogen solutions and have found that the carcinogen will penetrate the epidermis directly, even though the pilosebaceous apparatus is still undeveloped and penetration through the follicular openings thus cannot take place (*13*). It was of interest to investigate whether other organs that come into contact with carcinogenic hydrocarbons will also absorb them. Particular attention was directed toward the alimentary canal.

In our experiments we have tried to use chemically well-defined solvents for the carcinogenic hydrocarbons, especially those that are able to dissolve both water- and lipoid-soluble substances. We have, for example, used water-soluble polyethylene glycols (Carbowaxes) as carriers for the carcinogenic hydrocarbons in our experiments (*7–11, 13, 14*). The hydrocarbons dissolved in these compounds penetrate easily into the skin and induce cutaneous tumors. Carbowaxes in aqueous solutions are also suitable carriers for carcinogenic hydrocarbons—for subcutaneous injections, for instance.

The so-called association colloids furnished another type of both water- and lipoid-soluble solvents. These have the ability to bring carcinogenic hydrocarbons into clear and stable aqueous solutions (*1, 2*). Aqueous solutions of carcinogenic hydrocarbons will also induce cutaneous tumors (*3, 4, 12*), and tumors in the mouse forestomach (*5*), even when comparatively small quantities of the carcinogen are used.

The present communication deals with the penetration of benzpyrene, dissolved in Carbowax 1500, into the stomach wall of mice. The fluorescent microscope technique was used.

The animals used, about 75 in all, were adult mice of an anonymous, known strain employed for several years in our experiments on chemical carcinogenesis. 3 : 4-Benzpyrene dissolved in water-soluble Carbowax 1500 was introduced directly into the stomach of the animals by means of a stomach tube. The animals were killed immediately or 2–60 min or 24 hr after the application. The stomach (with or without preceding fixation in 10% neutral formalin solution) was cut on the freezing microtome at 10-μ thickness, and examined immediately with the fluorescent microscope (type Reichert Lux UV with a Philora-lamp HPW 125 w). Other specimens were embedded in paraffin in the usual manner and stained using the hematoxylin–van Gieson technique. Some unstained preparations were cut without prior application of the fluorescent substance. The concentration of 3 : 4-benzpyrene was 0.5%. (The investigative technique will be presented later in detail.)

The following results are reported:

Forestomach: Immediately after the application of benzpyrene in Carbowax 1500, the superficial keratinized layers showed a brilliant, almost dazzling, white fluorescence. All layers of the stratified squamous epithelium below these had taken up material with a strong blue fluorescence. The intensity of the fluorescence was much stronger than that seen in skin painted with the same solution. The fluorescent material was localized diffusely in the cytoplasm of all cells in all layers of the epithelium. Only the nuclei appeared optically empty. In other words, benzpyrene in this carrier immediately penetrates into the wall of the mouse forestomach (Fig. 1).

In addition, a strong blue fluorescence could be observed, almost without exception, in the region of both the circular and the longitudinal muscle layers of the forestomach. It was found that the fluorescent substance in this region of the stomach was gathered into a kind of fine network, which could be beautifully visualized with the fluorescent microscope (Fig. 1). We have not attempted to prove that this network of channels which

FIG. 1. Unstained frozen section from the wall of the mouse forestomach photographed in ultraviolet light. The keratinized layers, as well as the stratified epithelium, are strongly fluorescent (upper part of figure). In the muscle layer a network of fine channels contains material with blue to blue-violet fluorescence (lower part of figure).

contained strongly fluorescent material is actually a part of the lymphatic system, but it probably is. The very fine and well-developed network of channels, partly parallel and anastomosing, begins "rootlike" at the interface between the subepithelial loose connective tissue and the individual circular muscle bundles, and then runs plexiform toward the longitudinal muscle layers, and extends into the fine, longitudinal channels in this part of the stomach wall (Fig. 1). The network of these (probably lymphatic) channels extended continuously through the whole stomach—i.e., this fine system with strongly fluorescent material was well developed also in the glandular part of the stomach, as well as in the small intestine (at least in the upper segment). The mechanism of drainage of the fluorescent material via the channels mentioned above does not seem to have been observed previously.

Glandular stomach: When the preparations were made immediately after the introduction of the carcinogen dissolved in Carbowax 1500, the following could be observed: Although there is a so-called ridge at the boundary of the nonglandular and glandular part of the mucous membrane (at which the stratified cornified squamous epithelium sharply changes into glandular tissue), there did not seem to be great differences in the ability of cells in these two parts to take up fluorescent material. On the contrary, the gland cells had taken up large amounts of blue fluorescent substance in their cytoplasm. The nuclei were devoid of this material. The epithelial gland cells containing mucine had also taken up fluorescent material. The intensity of the fluorescence was sometimes as strong as that in the squamous-celled epithelium of the forestomach (Fig. 2).

Also, in this part of the mouse stomach, the (lymphatic) channels contained blue fluorescent material, and even the finest ones could be plainly observed under the fluorescent microscope.

The entrance and localization of the carcinogenic hydrocarbon (or its fluorescent metabolites) in the glandular part of the mouse stomach have not been established earlier with certainty. We have, however, found that benzpyrene dissolved in the water-soluble Carbowax 1500 (which is both water- and lipoid-soluble) immediately enters also the cells of the glandular stomach. Not only are the cells of the upper third of the tubules able to take up fluorescent material, but also the cells of the middle third (and sometimes the cells of the lower third) take it up in their cytoplasm. The lacteals also contained—at least to some extent—the fluorescent substance.

We have thus found that 3 : 4-benzpyrene dissolved in the water-soluble polyethylene glycol (Carbowax) very rapidly enters the cells of all segments of the mouse stomach—i.e., the wall of the forestomach as well as that of the glandular stomach, penetrating the "protective mucous barrier" of the latter. In addition, it (or its fluorescent metabolites) rapidly passes through the mucous membrane into a system, most probably lymphatic, of fine channels in the outer layers of the gastric wall. Apparently the drainage via the channels presented above then takes care of the elimination of this fluorescent material. It has thus been possible to observe directly how a substance introduced into the stomach of mice is drained by this system. The existence of the especially well-developed and rich drainage system in the wall of the mouse forestomach seems to mean that this segment of the digestive canal may play a certain role in the gastrointestinal absorption.

Certain investigations concerning tumor induction in the mouse glandular stomach, planned along the new prin-

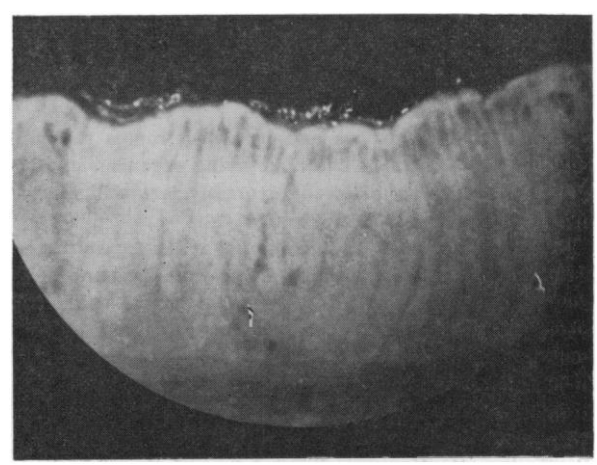

FIG. 2. Unstained frozen section from the wall of the glandular part of the mouse stomach photographed in ultraviolet light. Gland cells had taken up great amounts of strongly fluorescent material. Nuclei are optically empty.

ciples, are now in progress. The results here reported are preliminary to a report on a more detailed study of the significance of certain factors in experimental chemical carcinogenesis with carcinogenic hydrocarbons carried out since 1945 (*6*), as well as on the so-called solvent effect for chemical carcinogenesis in general.

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Oxygen Consumption and Radiophosphate Uptake by Minced Brain from Mice of Different Ages in Relation to Propagation of Mouse Encephalomyelitis Virus[1]

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It has been reported previously (*2*) that brain minces from mice up to 9 days of age are capable of supporting the growth of Theiler's GD VII strain of mouse encephalomyelitis virus when cultured in a simple medium containing only salts and glucose; brains from mice 1–2 days of age yield more virus than brains from mice 3–9 days old. No evidence of virus propagation was obtained with brains of mice 10 days old or more. In this study, we investigated the oxygen consumption and radiophosphate uptake of control and virus-infected brain minces from mice of different ages in an effort to determine whether metabolic differences might be associated with the inability of older tissues to support virus propagation.

The methods employed were described in detail in previous papers (*3*, *4*). Minced brain tissue (40–60 mg) was aseptically removed from mice of various ages and added to Warburg vessels or to 50-ml Erlenmeyer flasks containing 2.5 ml of Simms' solution. The pH was adjusted to 9 with dilute NaOH. Cultures were inoculated with virus or control supernatants and brought to a final volume of 3 ml with Simms' solution. Warburg experiments on oxygen consumption were carried out at 35° C with continuous shaking in an atmosphere of air. P^{32} uptake experiments were carried out at 35° C in stoppered, 50-ml Erlenmeyer flasks without shaking and in an atmosphere of air. At the termination of the incubation period all preparations were tested for sterility, and the virus titer was determined by intracerebral injection in mice with serial dilutions as previously described.

TABLE 1
OXYGEN CONSUMPTION BY MINCED BRAIN FROM MICE OF DIFFERENT AGES

Mouse age	No. of exps.	Virus present	Q_{O_2}* Initial	5 hr	12 hr	24 hr	Titer after 24 hr†
1 Day	10	–	90–110	60–80	40–60	30–40	–
	10	+	90–110	60–80	40–60	30–40	10^{-5}
3 Weeks	3	–	130–170	60–90	10–30	0	–
	3	+	130–170	60–90	10–30	0	10^{-2}
Adult	4	–	180–220	40–60	0	0	–
	4	+	180–220	40–60	0	0	10^{-2}

* Values of Q_{O_2} are expressed as µl O_2/hr/100-mg wet weight and show the ranges for the different experiments.

† The highest tenfold dilution that killed at least half of a group of 7 mice when 0.03 ml was injected intracerebrally. A titer of 10^{-2} represents only survival of the original virus added.

Oxygen consumption was measured on control and virus-infected tissues using the direct method of Warburg as previously described (*3*).

The results of experiments with minced brain from 1-day-old mice, 3-week-old mice, and adult mice are given in Table 1. The initial metabolic rate is distinctly higher in 3-week-old and in adult mouse brain than in the 1-day-old group. However, the metabolism of the older tissue declines much more rapidly with time than does that of 1-day-old brain mince. As was previously noted (*3*), the presence of the virus had no influence on oxygen consumption of the 1-day-old mouse brain, although the virus was shown to propagate rapidly in this tissue. No virus propagation was observed in 3-week-old or adult mouse brain.

Studies were made of the uptake of radioactive orthophosphate into the organic acid-soluble (OAS) fraction, the phospholipide (LP) fraction, and the "total protein-bound" (TPP) fraction by the procedures previously described (*4*). The chemical analysis for P^{32} was carried out by a modification of the method of Fiske and Subbarow (*1*) in which ascorbate was used as a reducing agent and a heating period was employed for color development and stabilization. Radioactive samples were prepared in ¼-oz tin ointment dishes, and

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