attended for long periods. If kept running continuously the supply carboys will have to be refilled about twice a week. Care must be taken they do *not* become empty, which would result in the flasks running dry and breaking, but this is the only matter that requires any attention. If the carboys can be filled directly from a central distilled water line without moving them, this can be done through a third inlet in the stoppers. In this case a stop-cock should be inserted in tube (c) so that flow of water into the distilling flask can be stopped while re-filling. Without this precaution water will continue to flow so long as the air-lock is broken in filling and the distilling flask may become flooded.

The total cost of the outfit is approximately $150. For small laboratories a single unit costing about $75 would be quite satisfactory. Some further saving can be made on the heating unit.

Two such units will distill about a liter of water an hour giving a product of high purity. With the heater, distilling flasks, tubes and supply carboys they occupy a space of 25 x 30 x 37 inches above the table.

Various refinements of this apparatus are possible. We know of at least 13 of these double units now in use. The design has proved highly satisfactory and is therefore presented with the hope that it may prove useful to others.

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## Incidence of Epidermal Methylcholanthrene Tumors in Mice After Administration of Cortisone.*† (20215)

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There appears to be some relationship between the skin irritation produced by application of carcinogenic hydrocarbons and the development of the ensuing tumors(1). There is also an undoubted effect of cortisone administration in reducing the inflammatory reaction of the skin in response to the application of various irritating chemicals(2-5). It seemed of interest, therefore, to study the possible effects of cortisone administration upon both the inflammatory response and the production of epidermal tumors in the skin of mice being subjected to applications of methylcholanthrene.

*Methods.* Albino mice of a Swiss strain, the same age and approximately the same weight, were used. *Group I-A.* 65 mice received applications of a 0.6% (weight/volume) solution of methylcholanthrene in benzene, applied to the clipped skin of the interscapular area 3 times weekly for 6 consecutive weeks; and each received, subcutaneously, concomitant daily injections of 0.5 mg of cortisone acetate in 0.25 cc of physiologic‡ saline solution. *Group I-B* (Control). 47 mice received paintings of methylcholanthrene-benzene as in Group I-A, but daily injections of 0.25 cc of physiologic saline without cortisone. *Group II-A.* 65 mice received applications of a solution of 0.3% methylcholanthrene in carbowax 1500 (weight for weight, solution complete at 41°C) 3 times weekly for 6 consecutive weeks and concomitant daily injections of cortisone acetate, as in Exp. I-A. *Group II-B* (Control). 47 mice received applications of methylcholanthrene in carbowax as in II-A, but concomitant daily injections of physiologic saline instead of the cortisone suspension, as in Group I-B. *Tumor formation* was regarded as established when a circum-

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scribed growth of the skin was observed to persist for 3 or more weeks. (Papillomatous growth on a pedunculated base, without surrounding infiltration, suggested the absence of malignancy; nodular growth on a broad base, with central depression and indurated margins, suggested the presence of malignancy.) The *degree of irritation* was judged by visual estimation of the intensity of erythema, the amount of scaling, and the onset of epilation. In a certain number of animals of each group, skin biopsies were made at regular intervals. The present paper contains a summary of the results bearing upon the degree of inflammation and of tumor development as apparently affected by the cortisone administrations and by the trauma of the excisions for biopsy. The results of the microscopic studies and other findings will be reported fully at a later date.

*Results.* By comparing Group I-A with I-B and Group II-A with II-B, it was seen that in the early phases of the experiment, the cortisone administrations resulted in some reduction of the skin erythema and in a delay (averaging 4 days) of epilation and desquamation in the experiment with methylcholanthrene in benzene, as well as in a less apparent reduction of the corresponding, though weaker, responses to methylcholanthrene in carbowax. This result was not unexpected.

Contrary to our expectation, however, the results obtained with cortisone were not in the direction of reduction, but rather in the direction of higher frequency in tumor development (per number of animals): as is shown by Table I,§ 41 of the animals, which had been painted with methylcholanthrene and had received concomitant injections of cortisone, developed tumors within the first fifteen weeks of the experiment. This figure represents 69% of the "effective total" (59) of animals, *i.e.* of the number of animals alive at the time of appearance of the first tumor in the experiment. This proportion of 69% of the cortisone treated animals compares with 48% (22 of 46 animals) in the control group, I-B. Similar differences were noted in the studies

§ We wish to thank Dr. Donald Mainland, Professor of Medical Statistics, New York University College of Medicine, for his advice and evaluation of our data.

TABLE 1. Incidence of Epidermal Tumors in Mice Exposed to External Applications of Methylcholanthrene, with and without Concomitant Injections of Cortisone.

| Group | No. of mice: | Wk of experiment 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | Totals | Total surviving mice without tumor by end of 15th wk | Total No. of mice in exp. | "Effective total No." of mice* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I-A | M in benzene with cortisone | – | – | – | – | 9 | 12 | 4 | 8 | 4 | 4 | – | – | – | – | – | 41 | 5 | 65 | 59 |
| | Dead without signs of t.g. | 1 | – | 3 | 2 | 3 | 1 | 2 | 2 | – | 2 | 2 | 1 | – | – | – | 19 | | | |
| I-B | M in benzene | – | – | 2 | 4 | 1 | 1 | 1 | 7 | 4 | – | 2 | – | – | – | – | 22 | 18 | 47 | 46 |
| | Dead without signs of t.g. | – | 1 | – | – | – | – | 2 | 2 | – | – | 1 | – | – | 1 | – | 7 | | | |
| II-A | M in carbowax 1500 with cortisone | – | – | – | – | – | – | – | 5 | 1 | 2 | 2 | 2 | – | 1 | 1 | 14 | 27 | 65 | 58 |
| | Dead without signs of t.g. | – | 1 | 3 | – | 2 | 1 | – | 1 | 1 | 1 | 3 | 7 | 1 | 1 | 2 | 24 | | | |
| II-B | M in carbowax 1500 | – | – | – | – | – | – | 1 | 1 | – | – | 1 | 1 | – | – | – | 4 | 28 | 47 | 45 |
| | Dead without signs of t.g. | 1 | – | – | – | 1 | – | 3 | 2 | 1 | 1 | – | 1 | 1 | 3 | 1 | 15 | | | |

* "Effective total No. of mice" represents No. of mice alive when first tumor observed. M = methylcholanthrene; t.g. = tumor growth.

with methylcholanthrene in the carbowax vehicle. As shown in our table, 14, *i.e.* 24% of the "effective total number" of animals in Group II-A (receiving cortisone) developed tumors during the first 15 weeks of the experiment, as compared with 4 of 45 animals, or 9% in the control group, II-B.

Our results suggest that there was an additional increase in the number of tumor-bearing animals in the groups which received cortisone and in which excision-biopsies were performed at intervals. This was apparent particularly in the experiment with the benzene solution of methylcholanthrene (but was noticeable also in the experiment with the carcinogen in carbowax). Thus, in Group I-A (methylcholanthrene in benzene plus cortisone administration) 9 of 10 mice (90%) which had excisions performed, developed tumors by the end of the 9th week of the experiment—as contrasted with only 28 tumor-bearing animals (57%) among 49 mice ("effective total") which had *not* been subjected to biopsies. On the other hand, 2 (25%) of 8 surviving mice which had been subjected to biopsies in Group I-B (control experiment with methylcholanthrene in benzene, without cortisone administrations) showed tumors by the end of the 9th week, whereas 47% of the animals ("effective total") in this group which were *spared* biopsy excisions, had developed tumors at that time.

*Summary.* 1. Under the conditions of our experiments, the administration of cortisone to mice given external applications of methylcholanthrene in benzene or in carbowax 1500 was followed by some reduction of the early inflammatory response and by an increase in the incidence of epidermal tumor formation per number of animals, as compared with the results in the control experiments without cortisone injections. 2. Biopsy excisions performed in the methylcholanthrene-exposed skin areas appeared to increase still further the incidence of tumor formation in the mice treated with cortisone.

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## Potassium Metabolism of Liver Mitochondria.* (20216)

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It has long been postulated that the active transport of potassium (K) or of other alkali metal ions such as sodium (Na) by living cells involves the intermediate formation of ion-carrier complexes(1). More recently, in various cells and tissues, analysis of the kinetics of K exchange by the use of radioactive tracer ($K^{42}$) has suggested that intracellular K is not homogeneous but that it may consist of separate fractions exchanging at widely differing rates(2,3). Although this finding could be interpreted as indicating the formation of an ion-carrier complex, the evidence is inconclusive and it has become essential to attempt direct observations at the sub-cellular level. Mitochondria were selected for study because aerobic phosphorylation has been implicated in K accumulation by kidney slices(4) and by retina(5); and because the capacity for catalyzing aerobic oxidation and for the aerobic generation of phosphate bond energy has been localized for the most part to this fraction of

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