were visible in the urine, which showed only a trace of albuminuria. This patient, and two others under 2 who showed no chemical or microscopical abnormalities in the urine or any suppression, developed a curious painless brawny oedema of the whole legs and arms. The tissues did not pit on pressure. The appearance of the extremities was such as is sometimes seen in children with a tendency to chilblains during cold weather. It appeared to be due to some form of circulatory disturbance. It was not due to cold, as the wards were well heated, and the condition passed off in all three cases within 36 hours of stopping the drug. All three children were acutely ill, and were being treated in oxygen tents. They had some cyanosis of the face, due to the primary disease. All three made a good recovery. We had not observed this condition previously in any patients treated with other sulphonamides. In one other case there was a transient haematuria without any crystal deposit and without diminution of urinary flow. The drug was continued in this case without any obvious ill effect.

Apart from these possible toxic effects, sulphamethazine was extremely well taken. Vomiting was entirely absent. Slight nausea in three cases did not necessitate stopping the drug. There was no mental disturbance or depression, which are such a marked feature of cases treated with sulphapyridine. Many of the patients developed good appetites during early convalescence and were able to take large amounts of food without inconvenience while still receiving the drug. The non-toxicity of the drug was especially noticeable with middle-aged and old patients. Cyanosis was not as pronounced as with other sulphonamides. Our conclusions so far are that this is the best drug we have used for pneumonia, especially for older patients.

Working in this hospital, Ormiston, Woodman, and Lewis (1942) had a very similar mortality rate in a similar series of 140 cases treated with sulphapyridine. In another series of 120 cases (Peters, Woodman, and Lewis, 1939) reported from this hospital the mortality rate was 9% with sulphapyridine. In the accompanying table we mean by "response" that the patient became afebrile and began to improve within the period stated.

Age Group	Response to Sulphamethazine in Days								No Response within Six Days		Total
	2 Days		3 Days		4 Days		5 Days				
	Br.	Lobar	Br.	Lobar	Br.	Lobar	Br.	Lobar	Br.	Lobar	
0-2	6	1	4	1	4	—	—	—	5*	—	21
2-12	6	3	9	4	5	1	1	1	—	—	30
12-40	2	3	—	3	—	—	—	2	1†	4	15
40 and over	1	2	—	2	—	4	—	—	—	2	11

* 2 died. † Died.

Summary

A series of 77 cases of pneumonia treated with sulphamethazine is reported, with a death rate of just over 4%.

The drug is extremely well tolerated at all ages, and appears to be remarkably free of serious toxic effect, especially in middle-aged and elderly patients. The results are as good as with sulphapyridine.

REFERENCES

- Ormiston, G., Woodman, D., and Lewis, F. J. W. (1942). *Quart. J. Med.*, n.s., 11, 155.
Peters, B. A., Woodman, D., and Lewis, F. J. W. (1939). *Med. Officer*, 62, 55.

Full details of the official forms of help available to local authorities in running publicity campaigns for the immunization of children against diphtheria are contained in a special brochure put out by the Ministry of Health, the Ministry of Information, and the Central Council for Health Education. This provides in handy form a complete guide for any local authority wishing to embark on, or intensify, an antidiphtheria campaign. It is pointed out that as the result of the vigorous efforts already made about 4,000,000 children have been immunized. But there are still over 4,000,000 children unprotected. The need for action, both national and local, therefore remains urgent if the aim of immunizing at least three out of every four children between the ages of 1 and 15 is to be achieved.

THE EFFECT OF INJECTIONS OF H 11 ON THE GROWTH OF MOUSE TUMOURS

BY

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In view of the account by Gye, Ludford, and Barlow (1943) of their experiments with H 11 on tumour-bearing mice a summary of tests with similar extracts which were carried out in this laboratory in 1941 and recorded briefly in the Annual Report of the British Empire Cancer Campaign, 1942, may be of value, particularly as the two neoplasms for the most part employed were different from those utilized by the above workers.

The first is a spindle-celled sarcoma, which was produced in 1934 by inoculation of dibenzanthracene into the hind leg of a mouse and has since been transplanted through 170 passages with a high percentage of successful grafts and a very small number of spontaneous regressions. Its characteristics and histology have been intensively studied (McDonald and Woodhouse, 1938), and it has been utilized in a number of investigations, including the anti-cancer properties of various chemicals (Annual Reports of British Empire Cancer Campaign, 1941, 1942), tissue-culture experiments (Jacoby, 1940), and inoculation experiments on the chorio-allantoic membrane of the chick embryo (Jacoby, McDonald, and Woodhouse, 1943).

The second type is represented by benzpyrene-induced skin epitheliomas which also have been employed on a considerable scale for testing the possible inhibitory effects of chemical and physiological substances. Spontaneous regression of these tumours is very infrequent, and the animals may survive in good general health for several months after the appearance of papillomas, so that therapeutic injections can be made over a long period. Moreover, this type of growth permits injection experiments to be started before active invasion of the underlying tissues has taken place—i.e., in the pre-malignant phase (see protocols 4 and 5)—and in a way seems more comparable to spontaneous tumours than to a grafted neoplasm after many passages.

A small number of mice inoculated with a strain of Carcinoma 63 were also therapeutically injected. This tumour material was derived from an animal kindly given by Dr. Ludford to my colleague Dr. F. Jacoby, and is presumably identical with the Carcinoma 63 described in the paper by Gye *et al.*

The Injection Material

The examples of H 11 used were: (a) A specimen kindly supplied by Mr. Thompson of the Hosa Laboratories described as of twice the concentration for normal use. (Designated in the protocols as "Hosa.") (b) Three extracts prepared according to the directions of Thompson and his collaborators (1941) from different samples of normal male urine. (Designated in the protocols as "Birmingham, 1, 2, 3.")

Animal Material

The total of tumour-bearing mice in 8 separate experiments was 104—43 with spindle-celled sarcomas (controls included), 19 with skin epitheliomas, and 22 with "Carcinoma 63" (controls included); 20 epithelioma-bearing animals served as controls for the "epithelioma test," some receiving injections of various chemicals and others no injections or saline only. Large and vigorous animals about 5 months old were used for the grafted tumours. The applications of benzpyrene had been begun when the animals were about 10 weeks old. Although the mice were not of a pure strain they were taken from the same stock of "albino-whites."

All the injections were made intraperitoneally and, in the case of the carcinoma-grafted animals, through the flank opposite to the site of the growth.

Results

A survey of the outcome of all the tests clearly indicates that the H 11 preparation has no inhibitory effect on any of

* Carried out for the British Empire Cancer Campaign, Birmingham Branch.

these tumours. The experiments with Carcinoma 63 (12 animals treated) conform exactly to those of Gye and his co-workers, and need not be reported in detail, and only the protocols dealing with the dibenzanthracene sarcomas and benzpyrene skin epitheliomas are given below.

It will be noted that, in experiment 2b, 6 animals were injected with H 11 on the same day as the grafts were introduced. In spite of this, four large tumours developed. Also, in experiment 5, minute warts, at probably a precancerous stage, were in no wise inhibited.

The sarcomas from two animals which had received injections daily for 16 days were examined histologically (experiment 3). Some central necrosis, such as is generally found in tumours at this period, was present, but there were many portions of normally staining sarcomatous tissue. Moreover, the tumours were still capable of vigorous growth, as was proved by re-inoculating fragments into three mice, two of which developed typical sarcomas. The microscopical appearance of a skin epithelioma from a mouse after 20 days' therapeutical injections (experiment 6) showed no material difference from sections of benzpyrene-induced epitheliomas in control animals.

Protocols of Animal Experiments with H 11

(1) 18 albino mice, average weight 30 g., inoculated with 155th sarcoma graft August 24, 1941. 16 definite tumours palpable August 29. 6 retained as controls. 10 injected twice daily with preparation Birmingham No. 2 until death of animal.

Results.—Controls: 1 tumour regressed by Sept. 29; remainder grew—period 16 to 35 days. Injected: 1 tumour regressed by Sept. 17; remainder grew similarly to controls—period, 8 to 28 days.

(2) 20 mice inoculated with 154th sarcoma graft August 6, 1941. 2 animals died, August 9 and 10. (a) 6 untreated, for control. (b) 6 injected H 11 ("Hosa" extract diluted with equal volume of water), starting August 6. (c) Ditto starting August 11.

Results.—(a) One graft—no take. One graft regressed by August 20. 4 large tumours developed; animals survived 30 to 40 days. (b) 2 grafts—no take. 4 large tumours after 24 to 68 injections—i.e., 12 to 35 days. (c) One graft—no take. 5 definite tumours after 26 to 80 injections—average duration 28 days.

(3) 12 mice implanted with sarcoma August 12, 1941. 11 successful takes visible August 17. 6 inoculated with H 11 "Hosa" double strength 1 c.cm. daily for 16 days. (One injected animal died after 10 days' treatment.) Killed with 5 controls Sept. 3 and legs with tumours removed. Combined weight of tumour-bearing legs of 5 controls, 15.3 g.; of 5 injected, 19.2 g.

(4) 6 mice with skin papillomas resulting from 5 months' application of benzpyrene solution in acetone. Applications suspended for 1 week before injecting with H 11 (Birmingham 1). These tumours were of different sizes and at various stages of "malignancy." Injections of H 11 for average of 43 days. In all cases the tumours grew, in several instances producing large keratinized masses which appeared by inspection to be at "malignant" stage at death of animal. This was confirmed in some cases by microscopical examination.

(5) 7 mice with papillomas selected at varying stages of development from small "wart" to medium-sized growth, 10 by 10 mm. Injected extract Birmingham 2 twice daily. All the tumours enlarged, and in instances in which two or more warts were present these fused into one large mass, 12 by 20 mm. Average injection time, 26 days.

(6) 6 mice with epitheliomas $7\frac{1}{2}$ by 10 mm. Injected 24 days with "Hosa" extract. Final size, 20 by 28 mm.; no instance of reduction in size. This corresponds to rapidity of growth in many series of control animals used in a variety of experiments.

Summary

In 54 mice bearing grafted dibenzanthracene sarcomas, Carcinoma 63, or induced benzpyrene skin epitheliomas no inhibitory effect on the tumour growth was found after prolonged injections of the urine preparation H 11 or similar extracts.

Two sarcomas removed from mice after such injections were successfully grafted into other animals, and histological examinations of treated tumours showed no difference from control material.

REFERENCES

- Annual Report of British Empire Cancer Campaign (1941), p. 121, London.
— (1942), p. 46.
Gye, W. E., Ludford, R. J., and Barlow, H. (1943). *British Medical Journal*, **2**, 65.
Jacoby, F. (1940). *Nature*, **146**, 301.
— McDonald, Stuart, and Woodhouse, D. L. (1943). *J. Path. Bact.*, in press.
McDonald, Stuart, and Woodhouse, D. L. (1938). *Ibid.*, **47**, 615.
Thompson, J. H., Holt, P. F., Forbes Jones, R., Haydu, N., and Kennedy, G. Y. (1941). *Med. Pr.*, **205**, 334.

COMPOSITE ZIEHL-GRAM STAINING METHOD FOR SPUTUM, PUS, AND EXUDATES

BY

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The majority of sputa sent to the pathologist for examination are accompanied by a request for tubercle bacilli and "organisms," or for "predominant organisms." Culture is usually not required, though this may be undertaken by the laboratory either for interest only or to confirm the presence or give more detail of the organisms seen by direct film. These specimens are examined by the Ziehl-Neelsen and Gram staining methods. At least two films must be prepared, stained, and examined. The same applies to pus from various sources—e.g., pleural cavity, glands, or kidney—where tubercle bacilli are suspected. Where large numbers of such specimens are examined daily, as in chest hospitals, big general hospitals, and public health "swab and sputum" laboratories, much time is spent in staining and examining the films. Moreover, the preparation of stains and slides occupies a great deal of the time of the technical staff. A single staining method which would show the presence of tubercle bacilli and "other organisms," both Gram-positive and Gram-negative, would be of great value. The more lengthy search for tubercle bacilli could then be made, while at the same time noting the presence and probable nature of other organisms. Further examination by culture or inoculation would follow as usual where indicated. Such a method, as used at the Brompton Hospital laboratory, is described below.

Technique of Method

Thin evenly spread films are essential, as three different colours are used; poor films—a disadvantage in any method—are useless. For this reason the method is not recommended for beginners, nor in laboratories where only a few specimens of this type are dealt with.

Fix the films by heat in the usual way.

The following stains and reagents are required: (A) Carbol-fuchsin: a 1% solution of basic fuchsin in 5% aqueous phenol (as for Ziehl-Neelsen). (B) Picric acid decolorizer: saturated solution of picric acid in ethyl alcohol, 5 parts; acetone, 1 part. Keep in a well-stoppered bottle or a staining trough with well-fitting cover. (C) Crystal violet: 0.25% aqueous solution. (D) Gram's iodine. (E) Acetone. (F) Auramine green counterstain.

Only one new stain is used. This I have called "auramine green." It consists of a combination of two basic dyes—auramine, an orange-yellow stain, and China green (or malachite green), which in solution gives more of a blue than a green colour. Gurr's reagents are used. The stain is prepared as follows:

Make 1% solutions of auramine and China green in distilled water. Shake occasionally for 24 hours. A deposit will be obtained with the former; this may be filtered off either before or after combination of the two stains. Alternatively, the supernatant stain may be pipetted off after standing. Mix in the proportion of 9 parts of auramine to 11 of China green and filter if necessary. Alcohol is not needed for solution. The mixture keeps well for several months with occasional filtering.

Flood films with A and heat to "near boiling" on the slide. Stain for 5 minutes or more. Wash off stain with water. Add B. Renew after 2 to 3 minutes. The average sputum film will require 4 to 6 minutes; but thin films, such as those from swabs—e.g., from a fistula in ano—will not need a further addition of decolorizer. (Alternatively, a dish may be used in which the slides may be immersed until just decolorized.) Wash thoroughly in water. Flood films with C—1 minute. Wash in water. Add D—1 minute. Decolorize with acetone. Wash quickly in water and apply F (auramine green counterstain)—15 to 30 seconds. Do not leave the stain on too long or overstaining of the background may occur and differentiation of the organisms be rendered less clear. Wash in water, blot, and dry.

The picture is as follows. Tubercle bacilli are stained crimson, with their beading showing as black dots at intervals