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.

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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

along the bodies of the organisms. This feature is more obvious in older specimens. *Myc. smegmatis* is decolorized and takes up a greyish-green colour, losing some of its Gram-positive character when treated with acetone. Thus urine sediments or pus from the urinary tract are suitable for examination by this staining method. Gram-positive organisms such as pneumococci and staphylococci appear violet-black, as in the usual Gram stain; while Gram-negative organisms such as *N. catarrhalis* and Friedländer's bacillus are grass-green in colour. The background of fibrin, mucus, and debris takes up a light-green tint, while cells vary somewhat according to their type and age. Thus epithelial cells show bluish-green nuclei with a light yellowish-green cytoplasm. Polymorph leucocytes, if not degenerate, show a deeper staining of both nuclei and cytoplasm, the latter being paler. Old degenerate pus cells take up a more uniform pale-green stain with more or less differentiation between nuclei and cytoplasm. Lymphocytes show a deep bluish-green nucleus, while endothelials appear more mottled, with a paler cytoplasm. As in the ordinary Gram stain, old degenerate Gram-positive organisms take up the counterstain and appear Gram-negative.

Incidentally, auramine green counterstain has been found useful in the direct Ziehl-Neelsen technique in place of methylene blue, and also in Gram's method in place of neutral red or safranin, both for pus smears and films from culture. The colour is restful to the eye (as noted by Mackie and McCartney (1942) for malachite green in the former method). In my opinion, however, the cellular appearances are rather clearer when methylene blue is used, and where this feature is of importance the latter stain is recommended.

REFERENCE

Mackie, T. J., and McCartney, J. E. (1942). *Handbook of Practical Bacteriology*, Edinburgh.

Medical Memoranda

Blast Perforation of Ear-drums

The following short notes on 60 cases of blast perforation of the ear-drums occurring simultaneously among Indian troops in the Middle East following a land-mine explosion may be of enough topical interest to merit publication.

HISTORY OF CASES

All these men were within fifteen yards of the mine, and some as near as five yards. Casualties were admitted to hospital within three hours of the explosion, and the ears of all 82 immediate survivors were examined, when 60 were found to have traumatic perforation of one or both tympana. In all, 100 drums were perforated, 50% in the antero-inferior quadrant, the remainder showing either a posterior perforation or a horseshoe perforation involving the greater part of the membrane. Tuning-fork tests revealed a middle-ear deafness in each case, and, although sepoys are not very suitable subjects for such tests, the majority of them showed a diminution of absolute bone conduction also for several days after the accident.

Preliminary treatment in all cases consisted in the insufflation of sulphanilamide powder, followed by packing the ear with sterile gauze. Nine ears were occluded with wax and were left severely alone until three weeks after the injury. Only three of these nine drums showed a perforation at the end of that time. Of the two cases in which syringing was employed to remove wax both became infected, although they dried up subsequently within a fortnight. The proportion of drums infected was 38%, four of which were classed as severe infections, while two of these required a cortical mastoid operation two weeks after the accident. In both these cases the drum and the post-auditory wound were dry and had healed five weeks after operation. All infected cases received sulphapyridine, 1 gramme 4-hourly for five days, and many of the cases were put on sulphapyridine for their other injuries.

All but four drums were dry within four weeks; but as it was impossible to keep all the patients in hospital until their drums had healed the percentage of complete recovery is not available; 45% were healed within four weeks.

CONCLUSIONS

The antero-inferior quadrant is the commonest site of injury. The proportion of infection to be expected under the most ideal conditions is 35 to 40%.

Insufflation of sulphanilamide powder is probably of value in preventing infection.

Deafness is of the middle-ear type, superimposed upon a temporary nerve-deafness.

The subsequent damage to hearing is not marked.

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Reviews

LECTURES ON MEDICAL ETHICS

Medical Ethics. By Rupert M. Downes, M.D., M.S., F.R.A.C.S. Based on a course of lectures by Dr. A. V. M. Anderson at Melbourne University. (Pp. 78. 5s.) Melbourne: Published by Ramsay (Surgical) Pty., Ltd., 340, Swanston Street. Obtainable at all booksellers.

This modest volume may certainly be commended to the junior practitioner as he enters on the responsible duties of his profession. It offers information and advice relative to positions which are met but imperfectly in the technical medical curriculum but are none the less important in the doctor's life and conduct as these are related to his colleagues, to his patients, and to the State. The advice in all the circumstances contemplated is excellent in itself and is based throughout on the recognized traditions of the profession. The author's persuasive style gives the book an attractive literary quality. If the subject offers little opportunity for originality in substance the manner in which the teaching is presented is both pleasing and effective.

There is no attempt to cover all the situations included in the department of forensic medicine. Rather the purpose is to explain and justify professional obligations which arise less from rigid rules than from personal opportunities and seemingly customs and courtesies. In this respect the book is both comprehensive and well balanced. If we may suggest an addition it would be a paragraph on the value of personal insurances as a wise protection against the risks and contingencies which are part of the common lot.

The author acknowledges his indebtedness to the well-known textbook of the late Dr. Saundby, but he is mistaken when he suggests that there are not more recent counsellors in the field. Two limitations may be noted. First, not all legislation affecting medical practice in Australia—for example, the compulsory notification of venereal disease—applies in Great Britain; and, secondly, the book wants, and the anxious inquirer desires, an index.

NUTRITION OF MOTHER AND CHILD

War-Time Food for Mother and Child. By Geoffrey H. Bourne, D.Sc. (Pp. 78. 2s. 6d.) London: Oxford University Press. 1943.

In less than 100 pages the author has succeeded in simplifying the nutritional problems that beset expectant and nursing mothers and their offspring. There are chapters which deal lucidly with the difficult periods in a child's life—infancy, kindergarten, and school. The advice given on weaning is excellent and should prove most helpful to the harassed mother. Since the publication of this book official action has been taken to supplement the maternal diet with cod-liver oil and vitamin C. However, there are still women who do not know that capsules of the oil are available, who have difficulty in obtaining them, and who think that liquid paraffin is just as beneficial. A wise dispensation of the Ministry of Food, announced by Lord Woolton, is that from July 25 the expectant mother is to be entitled to two ration books, the additional one enabling her to obtain supplies of food beyond her normal adult ration.

On page 29 the advice about eating cheese seems to be out of its context: there is little iron in this food. The author has rightly stressed the importance of an adequate intake of vitamin C during lactation. He points out that dilution of cow's milk with cereal waters like barley and oatmeal and with limewater is no longer supported by medical opinion. Sugar in excessive quantities for the pre-school child is condemned. It is a pity the "Oslo breakfast" has not been described in more detail. Dr. Bourne asks what has happened to the health sandwiches introduced by those perspicacious medical officers of health, Drs. Milligan, French, and Hunt. These sandwiches were excellent supplements for children, and the author would like them provided throughout the country. We must agree that even in the "best" boarding schools diets are provided which do not contain all the essentials. We would go further, and include hospitals, both voluntary and municipal, in this indictment; and it is a good sign of the times that a