the cases. Moreover, in some of the sections the liver cells show bile staining, and there are appearances of the same kind as those seen in cases of toxic jaundice. These features suggest that the increase of bilirubin in the blood is due to interference with the polygonal cells, sometimes by poisoning, sometimes by damming back of the bile in the smaller bile ducts, and sometimes by both. The increase of bilirubin shown constitutes latent jaundice. In the few cases in which the fragility of the red cells was examined it was found to be normal; this strengthens the view that the jaundice is due to something in the liver and not to haemolysis.

Laevulose Test.

The basis of this test is that laevulose given to a normal person does not increase the blood sugar, whereas when the liver is functioning deficiently there is a characteristic increase in blood sugar. The dose of laevulose is given on an empty stomach, and the blood sugar estimated half-hourly for two hours.

I have divided the cases into two classes (Table IV) as in the van den Bergh test. In Tables V and VI, dealing

TABLE IV.

Hepatitis Marked or Moderate.		Hepatitis Slight or Nil.	
Condition of Gall-bladder.	Laevulose Test (Liver Function).	Condition of Gall-bladder.	Laevulose Test (Liver Function).
Pathological ...	Deficient	Pathological ...	Not deficient.
,, ...	Deficient	,, ...	Not deficient.
Control case ...	Deficient	,, ...	Deficient.
Pathological ...	Deficient	,, ...	Deficient.
,, ...	Deficient	Control case ..	Not deficient.
,, ...	Deficient	,, ,, ...	Not deficient.
,, ..	Deficient	,, ,, ...	Not deficient.
,, ...	Deficient	,, ,, ...	Deficient.
,, ...	Deficient	,, ,, ...	Not deficient.
,, ...	Not deficient	,, ,, ...	Not deficient.

TABLE V.—*Cases in which Hepatitis was Marked or Moderate.*

Type of Case.	Van den Bergh Test.	Laevulose Test (Liver Function).
Pathological gall-bladder ...	D.N.; I.P. (s)	Deficient.
Pathological gall-bladder ..	D.N.; I.P.	Deficient.
Control	D.N.; I.P. (s)	Deficient.
Pathological gall-bladder ..	D.N.; I.P.	Deficient.
Pathological gall-bladder ...	D.N.; I.P.	Deficient.
Pathological gall-bladder ...	D.N.; I.N.	Deficient.
Pathological common duct ...	D.P.; I.P.	Deficient.
Pathological gall-bladder ...	D.N.; I.N.	Not deficient.
Pathological common duct ...	D.P.; I.P.	Deficient.
Pathological common duct ...	D.N.; I.P.	Deficient.

See Table III for explanation of letters in Tables V and VI.

TABLE VI.—*Cases in which Hepatitis was Slight or Nil.*

Type of Case.	Van den Bergh Test.	Laevulose Test (Liver Function).
Pathological gall-bladder	D.N.; I.N.	Not deficient.
Pathological gall-bladder	D.N.; I.P. (s)	Not deficient.
Pathological gall-bladder	D.N.; I.P. (s)	Deficient.
Pathological gall-bladder	D.N.; I.P. (vs)	Probably deficient.
Control...	D.N.; I.N.	Not deficient.
Control...	D.N.; I.P. (vs)	Not deficient.
Control...	D.N.; I.P. (vs)	Not deficient.
Control...	D.N.; I.N.	Deficient.
Control...	D.P. (s); I.P.	Not deficient (haemochromatosis)
Control...	D.N.; I.P. (vs)	Not deficient.

respectively with cases in which hepatitis was marked or moderate and others in which it was slight or *nil*, the results of both tests are placed side by side. One case, which was thought to be an instance of haemochromatosis, should be omitted. It will be observed that there is a close parallelism between the results given by the two tests; on the whole the state of the liver is shown more accurately by the laevulose test. This came rather as a surprise. I do not wish to stress these findings unduly, because the number of cases is small. So far as I know neither of these tests has been checked by microscopic liver examinations prior to this series, and it may be that the condemnation which the laevulose test has suffered is due, not so much to it being an unreliable test of the state of the liver, as to the fact that a result which indicates hepatic deficiency does not necessarily receive clinical support. This may be because our present knowledge of what is going on in the liver purely from clinical observation is depressingly deficient, and not because the laevulose test is at fault.

I feel that some explanation is due for presenting so few cases; I would point out that it is extremely difficult for a single investigator to produce a large series; the work involved is very time-consuming, and it is not always easy to get the necessary collaboration at the time the material comes to hand; moreover, the patient has to be considered primarily, and in many cases I did not feel justified in prolonging the operation beyond the period that was absolutely essential, nor in subjecting the jaundiced patients to a possible risk of oozing from the cut liver, and so on. However, every series adds to the total contribution to this subject, and a sufficient number of cases have been reported, I think, to warrant the conclusion that hepatitis is associated in some way with cholecystitis, and also with many other intra-abdominal lesions. In regard to the existence and type of the hepatitis, my findings agree very closely with those of Graham; on the other hand, the bacteriology of my series differs from his in degree rather than in kind; on both these points they are quite different from what A. L. Wilkie describes.

The correlation of functional tests of the liver with microscopic examinations has opened up a new field so far as I am aware; with such a few cases at one's disposal any explanation of the observed phenomena must to a large extent be speculative. Nevertheless, the results seem sufficiently interesting and uniform on the whole to make it worth while extending this method of research.

I should like to thank Dr. A. L. Taylor and Miss Hickman, who did the microscopic part of this work; Professor McLeod and Miss Wheatley, who are responsible for the bacteriology; and Dr. Fowweather, who determined the liver function tests. Without their help it would have been impossible for me to have obtained the reliable laboratory information so essential to this kind of investigation.

CARCINOGENIC SUBSTANCES AND THEIR FLUORESCENCE SPECTRA.

BY

E. L. KENNAWAY AND I. HIEGER.

(Cancer Hospital Research Institute, London.)

(*With Special Plate.*)

THE results described here are the latest obtained in an investigation of the carcinogenic action of coal tar. It has been known for many years that cancer could be produced by certain industrial products (gas-works tar, shale oil) which had been exposed to high temperatures, and this process of formation of carcinogenic materials has been extended in the laboratory of the Cancer Hospital Research Institute by heating a variety of substances (acetylene, isoprene, cholesterol; human skin, muscle, and hair; yeast) of which every one yielded products which caused cancer when applied to mice. An attempt was then made to obtain cancer-producing substances at body temperature by reactions which might resemble more closely those occurring in the tissues, when cancer develops naturally, than do changes in a red-hot tube at 700° to 900°.

Schroeter[1] in 1920 described a mixture of high-boiling compounds formed when aluminium chloride acted upon tetralin (commercial tetrahydronaphthalene) at 30° to 40° C. We found that this material was carcinogenic; one of the earliest experiments with it yielded 8 cancers and 3 papillomas from 20 mice in 256 days. This mixture of high-boiling compounds will be referred to as "Schroeter" for the sake of brevity. In some preparations

E. L. KENNAWAY AND I. HIEGER: CARCINOGENIC SUBSTANCES AND THEIR FLUORESCENCE SPECTRA.

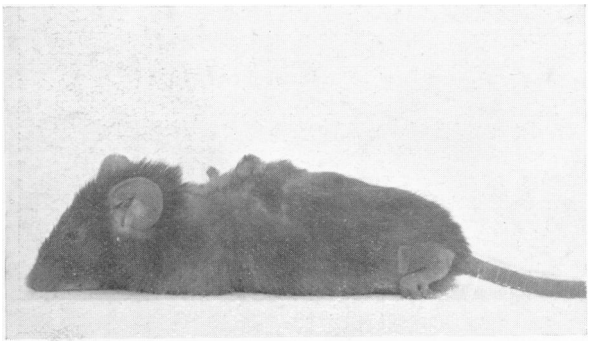


FIG. 1.—Mouse painted with 3′-methyl-1.2,5.6 dibenzanthracene. 205th day.

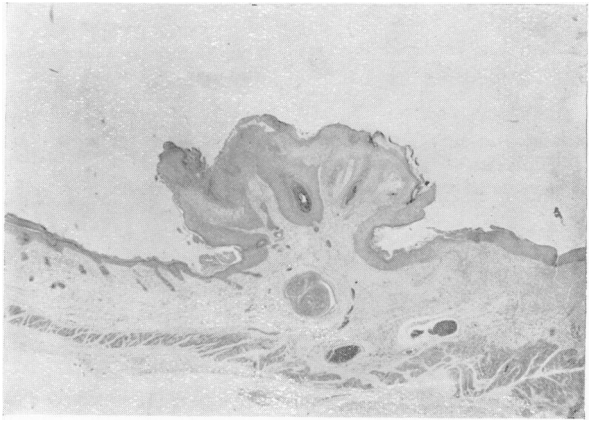


FIG. 2.—Papilloma. Mouse painted with 3′-methyl-1.2,5.6 dibenzanthracene. 189th day.

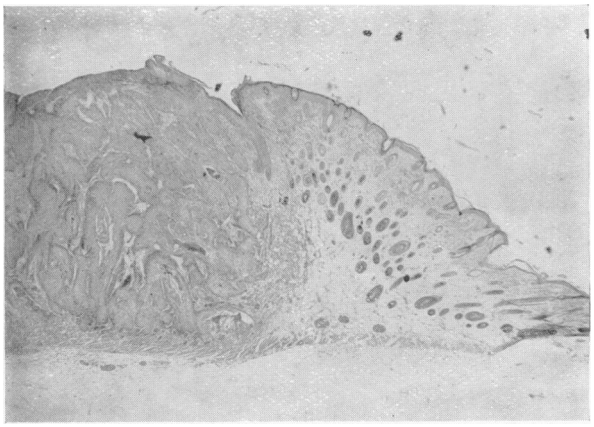


FIG. 3.—Epithelioma. Mouse painted with 1.2,7.8 dibenzanthracene. 223rd day.

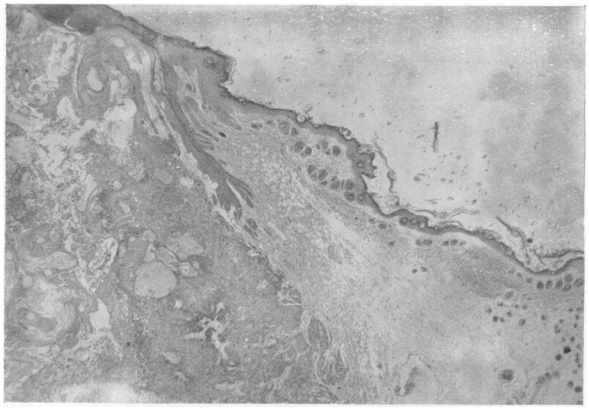
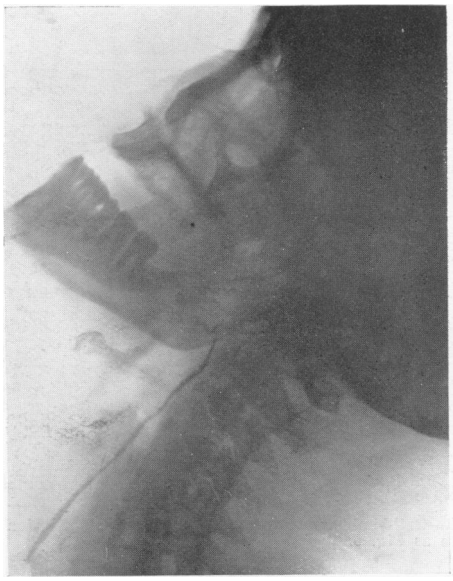
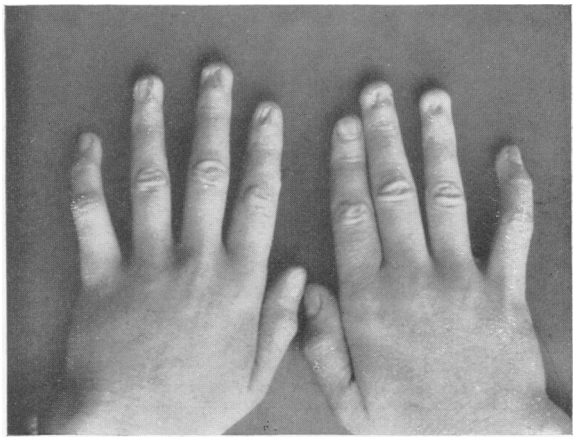


FIG. 4.—Epithelioma. Mouse painted with 1.2,7.8 dibenzanthracene. 252nd day.

A. H. G. JOHNS: PATENT BRANCHIAL CLEFT.



F. A. E. SILCOCK: CONGENITAL DYSTROPHY OF THE NAILS.



the rather higher temperatures (60° to 70°) used by Schroeter in his subsequent work were employed. Inactive material may be obtained if the aluminium chloride is in too low a concentration, or acts for too short a time. When these negative results are excluded the total yield of tumours from all the experiments with "Schroeter" which gave any tumours at all has been 118 cancers and 30 papillomas in 496 mice. Our later observations indicate that the carcinogenic agent may not be produced in *effective* amount at body temperature, but is increased to the concentration required to cause cancer in the skin of a mouse by the heating necessary to distil off the unaltered tetralin. Hence the original object of these experiments—namely, to produce an active carcinogenic agent at body temperature—may not have been attained, but the developments of this work described below have diverted our investigations for a time in other directions.

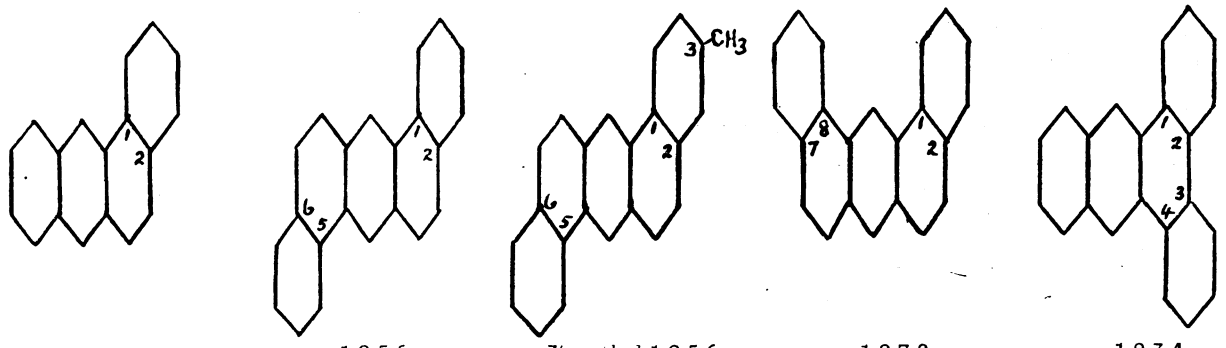
Tetrahydronaphthalene is not fluorescent, but the carcinogenic material obtained from it by the action of aluminium chloride shows a blue-violet fluorescence, which becomes very intense in a beam of ultra-violet light. If this fluorescent light is passed through a spectroscope on to a photographic plate a spectrum is obtained consisting of three bands of wave-lengths approximately 4,000, 4,180, and 4,400 A.U.; the bands are sharply defined on the short wave-length side and grade less abruptly into the background on the long wave-length side. The 3,680 A.U. mercury line is used as a fixed wave-length for comparison. A large number of pure hydrocarbons and other substances were then examined by the same methods in the hope of finding one which showed the spectrum of "Schroeter." No identical spectrum has as yet been found. However, one compound—namely, 1.2-benzanthracene—gives three bands which resemble those of "Schroeter" very closely, both in character and in position relative to one another, but in absolute position they lie nearer to the invisible region. Some reasons, which need not be set forth here, suggested that an addition to the benzanthracene molecule might produce the desired shift of the fluorescent spectrum towards the visible, and, possibly, the development of carcinogenic properties also. These additions may take various forms, such as:

1. The attachment to the hydrocarbon nucleus of side chains, which may be aliphatic (for example, the methyl group) or aromatic (for example, the phenyl or benzyl groups). A number of such compounds—namely, the 3-methyl-, 6-phenyl-, 9 : 10-diphenyl-, and 10-benzyl-benzanthracenes—have been synthesized in this laboratory for the first time by Dr. J. W. Cook, who will describe his methods in the *Journal of the Chemical Society*. Most of these compounds show a shift of the spectrum towards that of "Schroeter," but to not more than one-half of the desired extent. No one of them has as yet produced tumours in mice, but some of them have been under test for a very short time only.

2. The condensation of fresh benzene rings with the benzanthracene nucleus, which itself consists of four such rings. Four such five-ringed hydrocarbons—namely, the 1.2,7.8, the 1.2,5.6, the 3′-methyl-1.2,5.6, and the 1.2,3.4 dibenzanthracenes—were prepared in a fairly pure state and applied in benzene solution to mice, and the fluorescence spectra were examined. The 1.2.7.8 and both 1.2,5.6 compounds gave spectra of the "Schroeter" type, but with subdivision of one band; and the whole spectrum lies about half-way between those of benzanthracene and of "Schroeter." The 1.2,3.4 compound, which may be regarded as differing from the two others in that it is a 9.10 substituted phenanthrene, gives a spectrum of quite different type. The graphic formulae of the compounds are shown below.

1.2-Benzanthracene.

Dibenzanthracenes.



1.2,5.6 3′-methyl-1.2,5.6 1.2,7.8 1.2,3.4

Each of these four compounds was applied to ten mice; at the date of writing the experiment has lasted 285 days. The 1.2,3.4 series died out early, leaving one mouse only on the 133rd day, so that no comparison of this compound with the others is possible at present. A second series is under observation. One of the three survivors of the 1.2,5.6 series bears two well-developed papillomas.* Four mice in the 3′-methyl-1.2,5.6 series have produced papillomas. (Figs. 1 and 2.) In the 1.2,7.8 series, the first tumour appeared on the 169th day, when six of the ten mice were still alive; the animal was killed on the 223rd day and the tumour was found to be clearly malignant, showing invasion of muscle over a wide area. (Fig. 3.) Another mouse showed a papilloma on the 222nd day; the tumour grew rapidly, and when the mouse was killed a month later showed obvious invasion of muscle. (Fig. 4.) The last two survivors of the series, each bearing a tumour, were killed on the 278th day; both tumours were found to be malignant. Thus the ten mice painted with 1.2,7.8 dibenzanthracene yielded 4 cancers. The pre-cancerous period is longer than it is in mice painted with a good carcinogenic tar. Our experiments with the dibenzanthracenes will be repeated with larger series of mice and purer samples of the compounds. All such experiments require repetition with very pure material; those who have most experience of hydrocarbons will be the least ready to assert that any sample is quite pure. We have applied a large number of hydrocarbons (naphthalene, α–α–dinaphthyl, β–β–dinaphthyl, α–phenyl naphthalene, cyclohexane, cyclohexene, dodecahydrotriphenylene, acenaphthene, fluorene, anthracene, β-methyl anthracene, octhracene, phenanthrene, retene, truxene, naphthacene, tetrahydronaphthacene, chrysene, and others) to mice with negative results, and we have many other compounds now under test. Hence there are many controls upon the results obtained with the dibenzanthracenes. In the experiments of Twort and Fulton[2] chrysene produced 5 cancers and 10 papillomas in, apparently, several hundreds of mice (the exact number is not stated). The fluorescence spectrum of chrysene is very similar to that of benzanthracene in character, but in position is displaced considerably towards the region of shorter wave-length—that is, away from the "Schroeter" bands.

Apparently neither benzanthracene nor any of its derivatives has been found, and perhaps has not been sought for, in coal tar, and we are at present examining tar, and the products of the action of aluminium chloride upon tetralin, for such compounds. It is quite possible that there are, among the many compounds still undiscovered in coal tar, derivatives of benzanthracene which are far more powerfully carcinogenic than any known substances.

The "Schroeter" bands at 4,000, 4,180, and 4,400 A U. are shown most distinctly by the aluminium-chloride-treated tetralin, but they have been found also in the fluorescence spectra of the following carcinogenic materials.

*One of the tumours borne by the mouse in the 1.2,5.6 series has been found to be an epithelioma.

TABLE I.

1. Gas-works tar.
2. Creosote oil.
3. Green oil.
4. Anthracene oil.
5. Pitch distillate.

(2–5: derivatives of gas-works tar.)

6. Heated Californian petroleum.
7. Acetylene tar.
8. Yeast tar.
9. Muscle tar.
10. Cholesterol tar.
11. Hair tar.

If acetylene tar (No. 7) is saturated with chlorine, (*a*) the "Schroeter" bands are shifted about half-way towards the benzanthracene position, and (*b*) the carcinogenic power of the tar is very much lessened. In two comparable series of 70 mice the chlorinated tar produced 4 cancers and 4 papillomas, and the unchlorinated tar 21 cancers and 5 papillomas.

The following substances gave a diffuse spectrum covering very roughly the 4,000–4,400 region without differentiation into bands.

TABLE II.

1. Shale (blue) oil.
2. Pitch.
3. Gas-works tar (a sample made at 600°).
4. Spindle oil.
5. Refined lubricating oil.
6. Blast furnace tar.
7. Unheated Californian petroleum.

Of these, 1, 2, 3, and 4 are carcinogenic, and 5. 6. and 7 are not. The Californian petroleum, when heated to 880°, yields a strongly carcinogenic material (No. 6 in Table I). It is possible that in some or all of these cases the spectrum is confused by the presence of a number of fluorescent compounds.

The following materials gave the same spectrum as those in Table I, but have not produced cancer.

TABLE III.

1. Tetroyl propionic acid mother liquor.
2. Undistilled aluminium-chloride-treated tetralin.
3. Benzyl oleate (technical).
4. Oleic acid (technical).

Two possible explanations of this discrepancy may be suggested—namely (1) that the spectrum is given both by carcinogenic compounds and by allied compounds which are not carcinogenic, and (2) that the fluorescence test is a very much more delicate one than is the production of cancer in mice. Thus some hydrocarbons are fluorescent at dilutions of 1 in 200,000,000. One would not expect such solutions to affect the skin of a mouse. Twort has recorded papillomata in mice painted with oleic acid. Possibly heating of the oil in the process of manufacture is responsible for this effect.

Finally, to complete the list of exceptions, we must record one cancer produced by a non-fluorescent substance. One epithelioma occurred in 30 mice painted with commercial tetralin; we then painted 100 mice with pure tetralin, but obtained no more tumours.

The very frequent recurrence in cancer-producing agents of the fluorescent spectrum described suggests the possibility of the use of it for the preliminary examination of materials, such as lubricating oils, suspected of carcinogenic effect. The test on mice is, of course, necessary for conclusive proof.

All the spectroscopic work described here was carried out by I. Hieger under grants from the British Empire Cancer Campaign. We are indebted to Dr. E. de Barry Barnett for many specimens of pure hydrocarbons used for spectroscopic comparison, and to Dr. Alfred Piney for the accompanying photomicrographs.

REFERENCES.

[1] Schroeter: *Brennstoff-Chemie*, i, 39, 1920. [2] Twort and Fulton: *Journ. Path. and Bact.*, 33, 119, 1930.

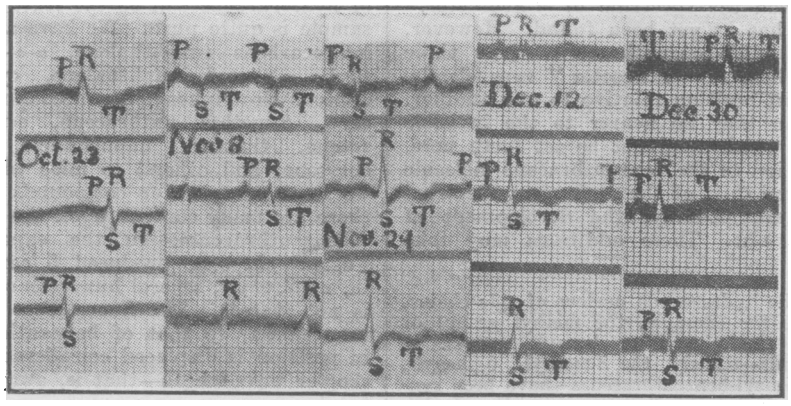
A CASE OF DIPHTHERIAL ACUTE MYOCARDITIS STUDIED BY MEANS OF THE PORTABLE ELECTRO-CARDIOGRAPH.

BY

DONALD HALL, M.A., M.D., F.R.C.P.,

PHYSICIAN, ROYAL SUSSEX COUNTY HOSPITAL, BRIGHTON.

In their paper in the *British Medical Journal* of January 18th, 1930, Dr. J. Strickland Goodall and Dr. T. Jenner Hoskin summarize the chief use of the electro-cardiograph as (1) diagnosis or cardio-analysis; (2) estimation of progress under treatment. The electro-cardiograms of the patient who is the subject of this short article were valuable, first, in establishing the diagnosis of acute myocarditis at a time when such could only be inferred from her symptoms in the absence of abnormal physical signs, and secondly, in giving a clear estimation of the condition of the myocardium as it became more severely affected, and then as it made a gradual but steady recovery from the acute inflammation.



The patient was a girl, aged 11, who was removed from the sanatorium of a boarding-school within the Brighton area, and was first electro-cardiographed on October 23rd, 1929, when I first saw her at the Brighton Borough Sanatorium in consultation with Dr. Graham-Bonnalie and Dr. C. Fraser Brockington, the resident medical officer. She was then in the twelfth day of diphtheria, and that morning had developed vomiting with precordial pain. So far as the circulatory system was concerned the only abnormality objectively was a low blood pressure (68/40 mm. of mercury). The cardiac borders and sounds were normal, and there was no interruption of rhythm. An electro-cardiogram, however, which was taken by the Cambridge portable instrument, gave us proof of myocardial affection. All the complexes, auricular and ventricular alike, were of low voltage. The Q R S waves were slurred, as well as stunted. The T wave in Leads I and II was shallow and inverted, while in Lead III the T was unidentifiable. The P waves, although small, were identifiable in all three leads, and the P-R interval was within normal limits. The heart's rate was 96.

She was electro-cardiographed again on November 8th, when she gave a record which is the most interesting of all. At that time there was clear evidence of an affected myocardium, the heart being dilated and rapid, with a canter rhythm; the blood pressure was still abnormally low, and particularly the pulse pressure: but as this is one of a series of cases in which Dr. Brockington has made a careful study of the changes of the blood pressure, which he intends to publish, I do not propose to go further into that matter. The electro-cardiogram showed the myocarditis of the left ventricle to be so great that the patient had a right-sided preponderance. The initial ventricular complexes were so low in voltage that they measured a mere 2 mm., but the initial complex in Lead I was an S and in Lead III an R, showing that for the time being the right ventricle was the predominating chamber. The T waves were much as before—in Leads I and II a very shallow inversion, and in Lead III hardly, if at all, identifiable.

On November 29th the electro-cardiogram had again changed. In Lead I the initial ventricular complex was a minute bizarre wave of the type met with in Lead III in a proportion of healthy subjects, but, in contrast to the previous record it contained both R and S elements. The T wave in that lead remained shallow and inverted. There was great alteration in the ventricular complexes in Leads II and III. Voltage had increased from 2 mm. to 11 mm., and there was a definite inverted T wave of ordinary type.

Further changes were noted in the electro-cardiogram of December 12th. In Lead I the small bizarre initial ventricular