It may at first sight seem to be unsatisfactory that 14 out of every 100 children operated on for suspected appendicitis will have a normal appendix and that two out of every five children admitted with this diagnosis will recover without treatment. These figures express, however, a widespread concern about the difficulty of diagnosing some cases of acute appendicitis in childhood. If the mortality of acute appendicitis is now very low, it must not be forgotten that a number of children still reach hospital gravely ill with peritonitis and survive only after much discomfort, hard work, and anxiety.

Readiness to admit and to operate on the doubtful case is the price we rightly pay for the earlier solution of the diagnostic problems which acute appendicitis in childhood will continue to set.

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Measurement of Digoxin in Plasma and its use in Diagnosis of Digoxin Intoxication

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Summary: A method for measuring the plasma-digoxin concentration uses the measurement of its inhibitory effect on ^{86}Rb uptake by human red cells in vitro. Patients receiving digoxin in whom there was no clinical evidence of digoxin intoxication had plasma digoxin concentrations ranging from 0·8 to 4·5 mμg./ml. Patients presenting with convincing clinical evidence of digoxin intoxication had plasma digoxin concentrations ranging from 4 to greater than 8 mμg./ml. It is suggested that the plasma digoxin concentration may be used as an aid in the diagnosis of digoxin intoxication.

Introduction

Cardiac glycosides inhibit the uptake of potassium by human red cells (Glynn, 1957). Love and Burch (1953) suggested the use of a rubidium isotope, ^{86}Rb, as a tracer for potassium in such metabolic studies because of its more convenient half-life (^{86}Rb, 19·5 days; ^{42}K, 12·4 hours). Lowenstein (1965) and Lowenstein and Corrill (1966) studied the inhibiting effect of plasma and extracts of plasma from patients receiving digoxin and digitoxin on the uptake of ^{86}Rb by normal human red cells in vitro, and demonstrated the feasibility of assaying the concentration of cardiac gylcosides in human plasma by such a method.

The purpose of the present investigation has been to develop this method further and to see whether the concentration of digoxin in plasma can be used as an aid in the diagnosis of digoxin intoxication.

Materials and Methods

Modified Krebs–Ringer phosphate solution was made up as follows: 0·9% NaCl, 1 l.; 1·22% $CaCl_2$, 15 ml.; 3·82% $MgSO_4.7H_2O$, 10 ml.; 0·1 M phosphate buffer, *p*H 7·4 (17·8 g. $Na_2HPO_4.2H_2O$ + 20 ml. *N* HCl diluted to 1 l.), 210 ml. 100 mg. of glucose was added to each 100 ml. of this solution before use.

Rubidium chloride ($^{86}RbCl$) was obtained from the Radiochemical Centre, Amersham, England, and diluted to 2·2 mM (initial specific activity 0.067 μmole/μCi) with Krebs–Ringer phosphate solution.

Preparation of Human Red Cells.—Venous blood was taken from normal subjects in a syringe rinsed with heparin (5,000 u./ml.). The blood was centrifuged at 1,000 g for 10 minutes at 5° C. and the plasma and top layer of cells were removed by suction. The red cells were then washed twice with ice-cold Krebs–Ringer phosphate solution by alternate resuspension and spinning. After the final spin the supernatant was removed and the packed cells were gently mixed and kept at 5° C. until used (not longer than four hours).

Extraction of Digoxin from Plasma.—5 ml. of heparinized plasma was vigorously shaken with 10 ml. of methylene chloride for two minutes in a stoppered glass tube and the tube centrifuged at 1,500 *g* for 10 minutes. The upper aqueous layer and the thick lipid interface were removed by suction. About 1 g. of anhydrous Na_2SO_4 was added to the remainder. The tube was vigorously shaken for 20 seconds and then briefly centrifuged. Of the clear methylene chloride 5 ml. was pipetted into a round-bottomed tube (16 by 125 mm.). The methylene chloride was evaporated to dryness in a stream of air at 45° C.

Incubation.—The dried extract was well mixed with 0·3 ml. of Krebs–Ringer phosphate solution and 0·2 ml. of prepared packed human red cells added. The suspension was incubated for 45 minutes at 36° C. with gentle shaking in air. Then 0·1 ml. of 2·2 mM $^{86}RbCl$ was added and incubation continued under the same conditions for a further two hours. The final volume of the incubation sample was 0·6 ml., and the concentration of $^{86}RbCl$ was 0·37 mM.

Preparation of Sample for Radioactive Assay.—At the end of the incubation 10 ml. of ice-cold 0·154 M NaCl was added with mixing. The sample was centrifuged at 1,500 *g* for 10 minutes at 5° C., the supernatant being removed by suction. The red cells were washed twice with 10-ml. volumes of ice-cold 0·154 M NaCl by alternate resuspension and centrifugation. After the final spin the supernatant was removed from the red cells as completely as possible. The packed red cells were then assayed for radioactivity in a scintillation-well system to detect the 1·08 meV γ-emission of ^{86}Rb at an efficiency of 10%. When the $^{86}RbCl$ was received the specific activity was

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0·067 μmole/μCi, and initially the radioactivity added to each sample was 3·3μCi. Though the specific activity declined with time, sufficient radioactivity was present at the end of five months for accurate assays to be performed with the stated concentration of ^{86}RbCl in the incubation samples.

Standard Dose-response Curves.—Each assay or batch of assays depends on the construction of a standard dose-response curve. The required amount of digoxin (Lanoxin), up to 40 mμg., was added in 0·1 ml. of 80% ethanol to 5 ml. of distilled water and the sample extracted, incubated, and assayed as described. Plasma from normal healthy subjects not taking digoxin was used as a control for the assay of plasma from subjects taking digoxin.

Results

Standard Dose-response Curves.—The variability of six dose-response curves for the inhibition of ^{86}Rb uptake by extracts containing known amounts of digoxin is shown in Fig. 1, where also is shown the method of calculating the plasma digoxin concentration in an unknown sample. Though the standard dose-response curves are all qualitatively similar there is an important quantitative variability which necessitates the setting up of a standard dose-response curve with each batch of unknown samples assayed.

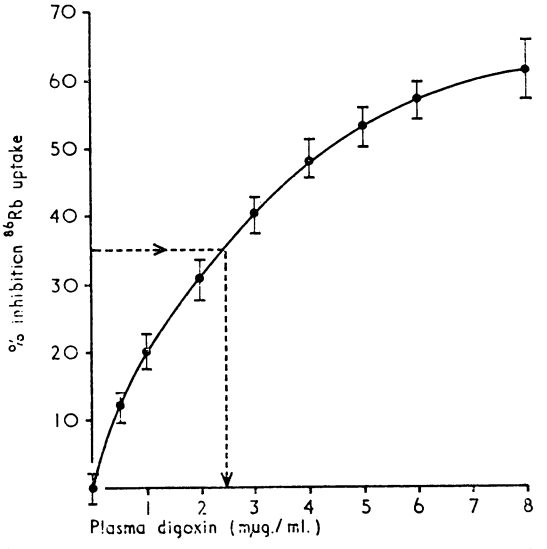

FIG. 1.—Standard dose-response curve. The curve is drawn through the mean values (bars represent S.E.M.) obtained from six consecutive standard dose-response curves. A hypothetical calculation of the digoxin concentration in a plasma sample is shown; the sample extract produced 35% inhibition of ^{86}Rb uptake by human red cells (see Methods), and this indicates a plasma digoxin concentration of 2·5 mμg./ml.

Plasma Digoxin Concentration and its Relation to Digoxin Intoxication.—The plasma digoxin concentration in subjects not receiving digoxin, in patients receiving digoxin in whom there was no suggestion of digoxin intoxication, and in patients in whom there were signs of digoxin intoxication are shown in Fig. 2. Only in one out of 15 subjects not taking digoxin did the plasma extract show any inhibitory effect on red cell ^{86}Rb uptake, and the equivalent plasma digoxin concentration of this sample was 0·4 mμg./ml. It should be stated that the assay as described here is not reliable for plasma digoxin concentrations less than 0·5 mμg./ml.

Forty-one patients taking digoxin 0·25 mg. or more per day in whom there were no symptoms or signs of digoxin intoxication had plasma digoxin concentrations ranging from 0·8 to 4·5 mμg./ml., with a mean concentration of 2·36 (S.E. ±0·16) mμg./ml. Nine patients studied had signs and symptoms suggestive of digoxin intoxication—that is, anorexia, nausea, vomiting, dizziness, confusion, headache, diarrhoea, or an arrhythmia. They had plasma digoxin concentrations ranging from 4 to greater than 8 mμg./ml. Four illustrative cases are briefly described below.

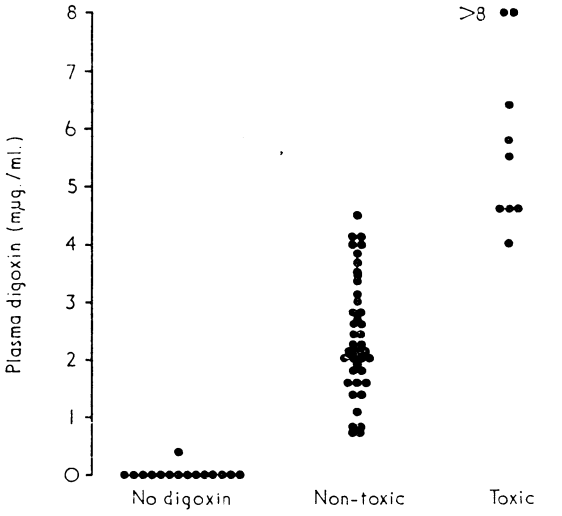

FIG. 2.—Plasma digoxin concentrations in subjects not taking digoxin (no digoxin), patients receiving digoxin who were not intoxicated (non-toxic), and patients receiving digoxin with clinical evidence of digoxin intoxication (toxic). In two toxic patients the plasma digoxin concentration was greater than 8 mμg./ml.

Therapy with spironolactone, chlorothiazide, ethacrynic acid, frusemide, methyldopa, guanethidine, bethanidine, and prednisone did not appear to interfere with the assay.

Illustrative Cases

Case 1.—Married woman aged 78. Diagnosis: hypertensive heart disease, deep venous thrombosis, pulmonary embolism, congestive cardiac failure, sinus rhythm. Initially "digitalized" with 2 mg. of digoxin given orally over 48 hours, and continued on digoxin 0·25 mg. b.d. Plasma digoxin concentration 13 days after digoxin began was 3·2 mμg./ml. At this time there were no symptoms or signs suggestive of digoxin intoxication. Eighteen days after digoxin was begun she complained of progressive anorexia, nausea, and dizziness. Plasma digoxin concentration at this time had risen to 4·6 mμg./ml. Plasma potassium was 3·9 mEq/l. Digoxin was stopped for two days and then restarted at a dose of 0·25 mg./day. Symptoms disappeared in 48 hours, pulse rate fell from 95–120 to 70–80 min. over four days, and the patient's general condition greatly improved. Electrocardiograms are shown in Fig. 3.

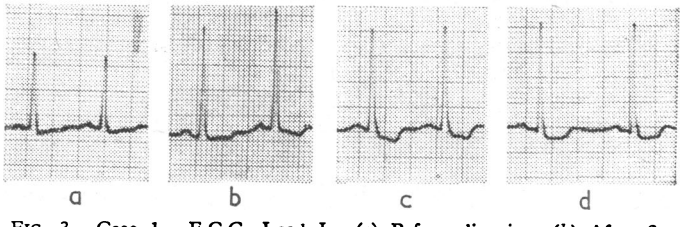

FIG. 3.—Case 1. E.C.G. Lead I. (*a*) Before digoxin. (*b*) After five days' digoxin therapy (see text). (*c*) After 13 days' digoxin therapy; plasma digoxin 3·2 mμg./ml. (*d*) After 18 days' digoxin therapy; clinically toxic; plasma digoxin 4·6 mμg./ml. Note progressive depression and sagging of S–T segment.

Case 2.—Married woman aged 76. Diagnosis: ischaemic heart disease, previous posterior myocardial infarct, recent anterior myocardial infarct, congestive cardiac failure, atrial fibrillation. Initially digitalized with 2 mg. of digoxin given orally over 48 hours, and continued on digoxin 0·25 mg. b.d. Two days after digoxin was started the plasma digoxin concentration was 2·4 mμg./ml. Progress was satisfactory until seven days after digoxin was begun, when she complained of feeling generally ill, and of postural dizziness. Pulse rate was 110/min., atrial fibrillation. Blood pressure lying 130/90, standing 50/?. Plasma digoxin had risen to 4·0 mμg./ml. Plasma

potassium was 4·3 mEq/l. Digoxin was stopped for two days and then continued at a dose of 0·25 mg./day. Within 24 hours she felt generally better, and the orthostatic hypotension had disappeared. Thereafter the ventricular rate was controlled at 70–80/min. on digoxin 0·25 mg./day. Electrocardiograms are shown in Fig. 4.

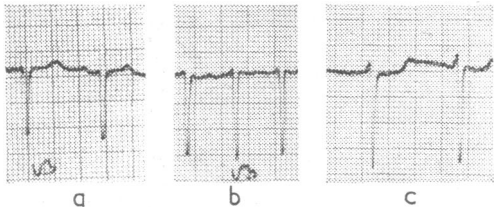

FIG. 4.—Case 2. E.C.G. Lead V3. (*a*) Before digoxin. (*b*) After two days' digoxin therapy (see text); plasma digoxin 2·4 mμg./ml. (*c*) After six days' digoxin therapy; clinically toxic; plasma digoxin 4·0 mμg./ml. Note increasing depression and sagging of S–T segment.

Case 3.—Spinster. Admitted for investigation and treatment of diverticulitis. For several years cardiac failure had been treated with digoxin 0·25 mg./day and diuretics. On admission to hospital digoxin 0·25 mg. b.d. was prescribed. After 23 days on this increased dose she complained of anorexia, nausea, vomiting, and diarrhoea. Plasma digoxin concentration was 4·6 mμg./ml. Plasma potassium was 3·8 mEq/l. Digoxin was stopped for three days, then restarted at a dose of 0·25 mg. alt. die. Her symptoms rapidly disappeared and the heart failure and atrial fibrillation continued to be well controlled on this dosage. Electrocardiograms are shown in Fig. 5.

Figure 5

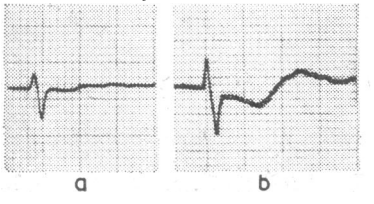

Figure 6

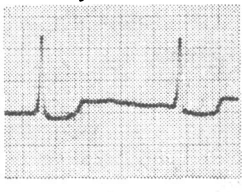

FIG. 5.—Case 3. E.C.G. Lead V4. (*a*) On admission, taking digoxin 0·25 mg./day. (*b*) After 23 days on digoxin 0·25 mg. b.d.; clinically toxic; plasma digoxin 4·6 mμg./ml. Note gross depression and sagging of S–T segment in *b*. FIG. 6.—Case 4. E.C.G. Lead V5. E.C.G. done when clinically toxic. Plasma digoxin 5·8 mμg./ml. Note depression and sagging of S–T segment.

Case 4.—Married woman aged 66. Diagnosis: ischaemic heart disease, congestive cardiac failure, atrial fibrillation. This patient had been on digoxin therapy (0·25 mg./day or b.d.) for five years. She was admitted in congestive cardiac failure, taking digoxin 0·25 mg. b.d., complaining of progressive anorexia, nausea, vomiting, and epigastric pain for one week, and severe frontal headache for one day. Pulse rate was 68/min.; atrial fibrillation. She had a previous history of presumptive digoxin intoxication, and the dose of digoxin had frequently been altered between 0·25 mg./day and 0·25 mg. b.d. On her admission the plasma digoxin concentration was 5·8 mμg./ml. Plasma potassium was 4·3 mEq/l. Digoxin was stopped for three days, then restarted at 0·25 mg./day. On this dose the symptoms rapidly settled, the congestive cardiac failure cleared, and the atrial fibrillation remained controlled. Electrocardiogram is shown in Fig. 6.

Discussion

Lyon and DeGraff (1967) were unable to reproduce the original procedure described by Lowenstein (1965) for the assay of plasma cardiac glycosides. Lowenstein and Corrill (1966) modified the method, and though the sensitivity was improved it was not fully brought into the range required for the reliable assay of plasma digoxin concentrations encountered during therapy. The modifications reported here increase the sensitivity some threefold to fivefold and allow the method to be used for measuring plasma digoxin concentrations at both the therapeutic and toxic levels, since the amounts of digoxin extracted from plasma may be compared with the full sensitive range of the standard dose-response curve.

The assay procedure, in its present form, has been set up specifically for digoxin. Because the plasma concentrations of other cardiac glycosides encountered during therapy differ from those of digoxin (Lowenstein and Corrill, 1966), the assay would have to be adapted to study a specific cardiac glycoside by altering the amount of plasma extracted and by constructing a dose-response curve for the cardiac glycoside under study. It is of interest that the range of plasma digoxin concentrations in non-intoxicated patients reported here does not greatly differ from that described by Lowenstein (1965) and Lowenstein and Corrill (1966).

It is generally accepted that myocardial binding is a prerequisite for the cardiac action of cardiac glycosides. The precise relationship between the plasma digoxin concentration and the amount of digoxin bound to the myocardium is unknown. In an individual patient the concentration of digoxin in the plasma reflects the interaction of several factors —that is, dose–weight relationships, absorption, distribution, metabolism, and excretion. Plasma protein binding might also be expected to affect the amount of digoxin available for myocardial binding. The final determinants of the therapeutic effect of digoxin must be the amount bound to the myocardium and the sensitivity of the myocardium to the drug. The wide spread of values for plasma digoxin concentration (0·8–4·5 mμg./ml.) obtained in patients accepted as being adequately digitalized and not intoxicated may reflect the interplay of these many variables.

But there is also great difficulty in defining the term "adequate digitalization." It is possible that some of the patients in the non-toxic group were taking doses of digoxin which might have been increased without producing toxicity and that the mean plasma digoxin concentration of this group (2·36 mμg./ml.) might be lower than if in each case digoxin had been purposely pushed to a just subtoxic dose. The electrocardiograms of 30 of the 41 patients in the non-toxic group showed S–T segment changes compatible with a digitalis effect, six showed no such effect, and in six we have no E.C.G., concurrent with the plasma digoxin estimation. The mean plasma digoxin concentration and spread of values observed in this group do represent those likely to be found in a routine clinical practice where patients are given maintenance digoxin therapy on the orthodox ad hoc basic of maintained clinical improvement. It was the intention in this study to compare the plasma digoxin concentrations of precisely such a non-toxic group of patients with those of intoxicated patients, and it is not the mean level of plasma digoxin concentration in the non-toxic group which is of importance in the diagnosis of toxicity but a concentration of 4 mμg./ml., above which digoxin intoxication becomes more likely. Further studies in which the clinical effects of digoxin and E.C.G. changes are correlated with plasma and tissue concentrations of digoxin will help to define more accurately what is meant by "adequate digitalization," particularly in patients with cardiac failure in regular rhythm.

The factors mentioned above are also relevant to the state of digoxin intoxication. Lowenstein (1965) showed that within one minute after the intravenous injection of digoxin 0·5 mg. the plasma digoxin concentration rises as high as 90 mμg./ml. but that distribution and tissue binding quickly occurs, so that within 90 minutes digoxin is undetectable in the plasma. Such a procedure in a patient not having previously received digoxin would not ordinarily produce digoxin intoxication. Yet on the basis of the results presented here a prolonged plasma digoxin concentration of 90 mμg./ml. in a digitalized patient certainly would be associated with toxicity. One must presume, therefore, that it is not the plasma digoxin concentration which is of primary importance in toxicity but the tissue concentration, the latter ultimately being determined by the former.

The proposed use of the plasma digoxin assay as an aid in the diagnosis of digoxin intoxication is based on the assumption

that during long-term therapy the plasma digoxin concentration reflects the tissue concentration because of a fairly predictable plasma: tissue distribution ratio. All the patients with digoxin toxicity reported here had been previously digitalized and intoxication occurred during the process of chronic therapy as a result of the slow accumulation of digoxin. In this clinical context it is possible to cautiously plan a therapeutic approach to the problem on the basis of the results obtained.

If an adult patient has been digitalized with digoxin and has been taking digoxin orally for more than three days, and presents with symptoms or signs suggestive of digoxin toxicity and the plasma potassium is not less than 3·5 mEq/l., then:

(1) If the plasma digoxin concentration is less than 4 mμg./ml., digoxin intoxication is very unlikely.

(2) If the plasma digoxin concentration lies between 4 and 5 mμg./ml., then digoxin intoxication is very likely and digoxin therapy should be temporarily stopped and restarted at a lower dose. Certainly increasing the dose of digoxin would be contraindicated. If in this situation emergency therapeutic measures were indicated, a fairly safe assumption of digoxin intoxication could be made and appropriate measures instituted.

(3) If the plasma digoxin concentration is greater than 5 mμg./ml., then a definite diagnosis of digoxin toxicity could be made.

The nine patients whose plasma digoxin concentrations are depicted in Fig. 2 as toxic have been handled according to these criteria with impressive clinical improvement.

In two of the illustrative cases (Nos. 1 and 2) sequential E.C.G. tracings are shown (Figs. 3 and 4) and though in these cases there are other possible causes of increasing S–T segment sagging these changes progressed as the plasma digoxin concentrations rose and the patients became intoxicated.

The measurement of plasma digoxin concentration would seem to offer a rational approach to the diagnostic problem of digoxin intoxication and helps in distinguishing those cases in which the presenting symptoms and signs might be due either to the intrinsic heart disease or to digoxin toxicity. The alternatives to a method such as that described here are double isotope derivative dilution assays, such as that described by Lukas and Peterson (1966) for digitoxin, and radioimmunoassay techniques as described by Butler and Chen (1967) for digoxin and by Oliver *et al.* (1968) for digitoxin. Double isotope derivative dilution assays take far too long to be of much use in a clinical situation, and the radioimmunoassay techniques seem to offer little advantage over the procedure described and are at present too complex to be of widespread applicability.

We are grateful to the physicians of St. Mary's Hospital who allowed us to study patients under their care, and also to the Endowment Fund of St. Mary's Hospital and Pfizer Limited for financial support.

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Cellular Viraemia in Babies Infected with Rubella Virus before Birth

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Brit. med. J., 1969, **1**, 289–292

Summary: Chronic viraemia has been detected in 10 out of 12 rubella syndrome babies at periods ranging from 1 to 196 days. The virus was found to be associated with leucocytes, and it is assumed that removal of neutralizing antibody is the most likely explanation for the high success rate in detecting viraemia. The findings are discussed in relation to diagnosis by virus isolation, to pathogenesis, and to the possible significance in explaining the failure of the foetus to develop a tolerance to rubella virus. Several published reports of viraemia in the acute exanthematous disease are contrasted with the less frequent reports of viraemia in the chronic disease of early postnatal life.

Introduction

The chronic excretion of rubella virus for the first two to three years after intrauterine infection is now well recognized (Phillips *et al.*, 1965; Dudgeon, 1967). However, despite recovery of virus from various tissues and excretions, isolation from blood has been reported only rarely.

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This paper describes rubella virus isolation from the peripheral blood of 10 out of 12 congenitally infected babies tested at ages ranging from 1 to 331 days. The demonstration of cellular viraemia is important not only as an adjunct to diagnosis, as it was in three patients, but also in relation to an understanding of the pathogenesis and the immunological aspects of the congenital rubella syndrome.

Materials and Methods

Patients.—Only 12 of the 33 virus-proved congenital rubella babies seen at this hospital in the past three years were studied for evidence of viraemia. They were selected in a random manner without regard to age or to degree of severity of their illnesses. The clinical course varied from patient to patient, but as these details are beyond the scope of this report case histories are not presented.

Virus Isolation.—The specimens examined varied with each patient, and though rubella virus isolation was attempted, usually from throat swabs, urines, and unclotted blood conjunctival swabs, lens aspirates and necropsy specimens were also tested. Cell cultures used for virus isolation included continuous rabbit kidney cell line (RK13, Glaxo; not freed of mycoplasma contaminants) and primary or secondary cultures of cynomolgus monkey kidney epithelium (MK). In the former