concentrated, as shown by a specific gravity of 1063 or over, even though the strength of the pulse may only have fallen a little below normal, as indicated by a blood pressure of not much below 100 mm. I have previously advised that intravenous injections should always be given if the specific gravity is extremely high, such as over 1065, but I would now go further and recommend this treatment to be adopted as soon as the specific gravity has risen to 1063 or over in the stage of acute copious diarrhoea, in order to avert the nearly inevitable collapse if they are withheld. If, however, the acute diarrhoea stage is passed, and the reaction stage with a rise of temperature has commenced, the blood pressure being over 80 mm., an intravenous injection is not necessary, while it is more likely to produce an excessive febrile reaction at this stage.

THE PREVENTION OF URAEMIA.

I have little to add under this heading, except to repeat that the further reduction in the death-rate from this complication must mainly depend on the earlier use of intravenous injections to prevent the onset of collapse with the accompanying stasis of the renal circulation, which is the essential cause of the subsequent deficient renal secretion. It has long been known that the frequency of uraemia is in proportion to the duration of the collapse stage. This point is further illustrated by the fact that all the fatal uraemic cases at Palermo were admitted on the second and third days of the disease, while all also showed a specific gravity of 1063 or over. Several patients admitted after two or more days' complete suppression of urine, and with a high specific gravity and threatened uraemia, were treated by slow intravenous injections (isotonic solutions being used if the blood pressure was nearly normal and the diarrhoea stage past), with the result that the urinary secretion was rapidly re-established and recovery from a very grave condition followed. If in this stage the specific gravity is not above normal, intravenous injections might produce oedema of the lungs, so in that case only subcutaneous and rectal salines should be given. If the respirations continue to be frequent and laboured after urine is being freely passed, oedema of the lungs should be sought for, and if present all saline injections should be stopped.

Such are the lessons I learnt at Palermo, and I venture to hope that their application in the future will do something to still further lessen the death-rate of cholera. That cases will still be met with of too virulent a nature, or coming too late under observation to be saved from a fatal termination, it is useless to deny. Nevertheless, these should be quite exceptional in previously healthy subjects, who are neither old nor extremely young, and who come under treatment at a fairly early period of the disease. In such I think the recovery-rate should be fully 80 per cent. However that may be, the results already obtained furnish a remarkable example of the successful application of modern methods of research, in discovering a life-saving method of treatment of one of the worst scourges which humanity is heir to, and one which should go far towards robbing the disease of much of its terrors in countries adequately provided with skilled medical men.

ADDENDUM.

Since the above sanguine estimate of the probable further reduction of the death-rate in cholera by the still earlier and more frequent use of the hypertonic saline treatment, as advised in the foregoing paper, was written, a report has reached me from Palermo which has more than fulfilled it. Professor Romano has sent me notes with blood pressure and specific gravity estimations of 54 severe cholera cases, all with specific gravities on admission of 1063 and over, which were treated by hypertonic intravenous injections during the month after I left Palermo, with only 8 deaths, or 15 per cent., two of the fatalities having been due to late pulmonary complications. After making every allowance for any possible decrease of the virulence of the disease, these are most remarkably successful results. The impression made by them on Professor Romano and his assistants may be gathered from the opening sentence of his letter, a translation of which reads as follows:

"I have the honour to inform you of the marvellous progress made by the patients whom you left under treat-

ment here at the time of your departure. I have delayed writing in order that I might further communicate, by means of the accompanying letter and forms, the results obtained from a large number of patients who were brought here since then in a hopeless condition, and who have been almost all saved through your valuable and miraculous method of treatment." The Professor goes on to say that he and his assistants hope to be permitted to go to Tripoli to spread the use of my methods there. This generous testimony can leave no possible doubt as to the value of the treatment advocated in this paper.

REFERENCES.

¹ *Cholera and its Treatment*, Oxford Medical Publications. ² *BRITISH MEDICAL JOURNAL*, September 24th, 1911. ³ *Proc. Roy. Soc.*, 1909. ⁴ *Indian Med. Gaz.* December, 1910.

THE PHYSIOLOGICAL ACTION OF AMORPHOUS DIGITOXIN (DIGALEN).

By W. LEGGE SYMES, M.R.C.S.,

DEMONSTRATOR OF PHYSIOLOGY, UNIVERSITY OF LONDON.

(From the Physiological Laboratory of the University of London.)

IN 1904 Cloetta¹ described a product obtained from digitalis leaves which he regards as amorphous digitoxin, and which is now in the market under the name "digalen." I am indebted to the manufacturers of this substance for samples for the purpose of physiological experiment.

Cloetta's own physiological work on digalen dealt chiefly with the question of cumulation. He confirmed Fraenkel's statement² that crystalline digitoxin may give rise to cumulative effects in cats, and added that animals which had exhibited cumulation with this drug showed none even with larger doses of digalen.³ Cloetta also found that digalen appears unchanged in the urine.⁴ Sasaki,⁵ testing digalen on the frog's heart, by subcutaneous injection and by directly douching the ventricle, observed characteristic digitalis effects.

Neave⁶ tested digalen, by perfusion, on the heart and blood vessels of the frog, and by intravenous injection on the rabbit. He also observed characteristic digitalis effects, adding that digalen is less active and also less irritant than either crystalline digitoxin or the *B.P.* tincture of digitalis. Worth Hale⁷ examined digalen toxicologically and physiologically. He found it less poisonous, and (on the circulatory system) less active than crystalline digitoxin. The lack of uniformity in his results emphasizes the desirability of obtaining digitalis preparations from manufacturing chemists rather than from drug stores. Both Neave and Hale employed doses which, judged by the following observations, were of toxic rather than of therapeutic order.

The present paper deals with two main sets of observations: (a) By intravenous injection, on fully anaesthetized cats; and (b) by perfusion, on the surviving heart of the frog.

Observations on the Cat.

In these anaesthesia was induced and maintained throughout the experiment by ether.

The solution supplied in "ampoules," which contains 0.3 mg. of digalen and 70 mg. of alcohol per c.cm., was (unless otherwise stated) diluted ten times with normal saline and injected slowly into the saphenous vein, whilst blood pressure was recorded either by a mercurial or by a membrane manometer. Urine was collected and measured, both before and after the injections, at intervals of ten minutes, by means of a catheter left continuously in the bladder.

Intravenous injections, containing 0.1 to 0.4 mg. of digalen, produce characteristic digitalis effects—namely, slowing of the pulse, augmentation and prolongation of the systolic wave, increase of arterial pressure, and increased secretion of urine. Such effects usually commence to subside about half an hour after the injection.

The slowing of the pulse by such doses is rarely great, occasionally zero. The normal pulse of etherized cats (200 to 250 a minute) is generally slowed by 10 to 30 beats

a minute. The change in the systolic wave is shown in the following figures :

Fig. 1.—Pulse tracings with Hürthle manometer. Weight of cat = 2.4 kg.; 0.15 mg. digalen (in 12.5 c.cm.) injected into the saphenous vein at 2.36 p.m. The figures on the right of each tracing show the scale of the manometer in mm. Hg. The time tracing indicates seconds. Pulse-wave increased in amplitude, diminished in frequency. Arterial pressure raised.

The next figure shows the effect of a larger and more concentrated dose. The amplitude of the systolic wave is reduced and the mean arterial pressure only slightly increased.

Fig. 2.—Pulse tracings with Hürthle manometer. Weight of cat = 2.0 kg.; 0.3 mg. digalen (1 c.cm.) injected into saphenous vein at 2.6 p.m. The figures on the right of each tracing show the scale of the manometer in mm. Hg. The time tracing contains one interval of one second in each case.

The effect on arterial pressure is variable, excessive as well as minute doses producing less increase than do doses of intermediate magnitude. Doses of 0.4 mg. and upwards

lead at times, as noticed by Hale, to some disturbance of the respiration. This is never great, and is absent even with doses which, kilo for kilo, are far in excess of those administered to the human subject.

Fig. 3.—Effect of digalen on arterial pressure and on respiratory movements: 0.1 mg. of digalen (3 ampoule) in 3 c.cm., injected into saphenous vein at arrow. Blood pressure recorded by mercurial manometer connected to left carotid artery. The respiratory movements (abdominal) are not affected by the dose. Weight of cat = 2.5 kg. Time tracing shows minutes.

TABLE I.—Effect of Digalen on Arterial Pressure of Etherized Cats.

	Weight of Cat in Kilos.	Time.	Dose.	Dose per Kilo.	Mean Carotid Pressure in mm. Hg.	
					Before Dose.	After Dose.
1	2.5	3.22 p.m.	0.1 mg.	0.04 mg.	163	170
		4.6 "	0.3 "		134	158
2	2.8	3.10 p.m.	0.1 mg.	0.036 mg.	124	140
		3.30 "	0.2 "		64	88
		3.55 "	0.3 "		56	96
3	2.3	2.56 p.m.	0.4 mg.	0.17 mg.	140	176
		4.2 "	0.5 "		94	108

In the second cat the brain and spinal cord were completely pithed between the first and second doses. In the third cat the greater dose produced the smaller increment in blood pressure (cf. Figs. 1 and 2). The normal mean carotid pressure of an etherized cat is 150 to 170 mm. Hg.

Additional instances of blood-pressure effects are given in the above table. These increments exceed those described by Neave (etherized rabbits) and by Hale (cats under urethane and chloral). Neave found that doses of 1 c.cm.* and of 2 c.cm. (= 0.3 mg. and 0.6 mg. of dry digalen respectively) produced scarcely any effect, whilst 4 c.cm. raised blood pressure from 84 mm. Hg to 96 mm. Possibly some of the difference may be attributable to the difference in animals, but in the light of the above table the doses are excessive. Hale used 2 c.cm.* (0.6 mg. of dry digalen) as the routine dose, repeated it after half an hour, and apart from this excessive dosage employed an obviously undesirable anaesthetic—namely, chloral.

Both Neave and Hale found that digalen has, weight for weight, less effect on the circulatory system than has crystalline digitoxin, and the former observer regards its solution, volume for volume, as less active than the official tincture of digitalis. As far as can be judged from the few trials of the latter preparations, made in the course of the present work, digalen is less active than both of them. But the difference is largely counterbalanced by the smaller irritation produced by injections of digalen (Neave). Further reference is made to this point below.

The diuresis produced by intravenous injection of digalen is comparable with that produced by crystalline digitoxin and by tincture of digitalis. This effect of digitalis preparations intravenously injected is of comparatively brief duration,* subsiding, as stated by Brunton and Power,⁹ less rapidly than the increment in arterial

* Whether from bottle or from ampoule is not stated.

pressure. Table II presents instances of such diuresis. In each case where a second dose was administered the effect of the first had largely passed off.

TABLE II.—Effect of Digalen and of other Digitalis Preparations on the Secretion of Urine.

	Weight of Cat in Kilos.	Dose.	Average Volume of Urine per Hour.		Duration of Observation.
			Before Dose.	After Dose.	
A	2.4	0.3 mg. digalen* 0.3 mg. digalen	10.5 c.cm.	16.7 c.cm. 16.5 "	20 min. 40 "
B	2.1	0.1 mg. digalen 0.2 mg. digalen	7.5 "	15.7 " 16.5 "	20 " 40 " 20 "
C	2.0	0.3 mg. digalen	3.0 "	13.8 "	20 " 50 "
D	2.4	0.3 mg. digalen 0.1 mg. digitoxin†	5.0 "	15.0 " 15.0 "	30 " 60 " 60 "
E	Not noted	0.15 mg. digalen 0.1 mg. digitoxin†	4.0 "	9.6 " 6.5 "	30 " 160 " 70 "
F	2.6	0.1 mg. digalen	4.5 "	16.9 "	40 " 80 "
G	2.6	$\frac{1}{2}$ c.cm. tinct. digitalis; Ditto	18.0 "	25.2 " 28.7 "	30 " 100 " 90 "

* Hoffmann La Roche Co. † Merck. ‡ Willows, Francis, and Butler.

Each 0.1 mg. digalen implies 23 mg. of alcohol.

Each 0.1 mg. digitoxin implies 175 mg. of alcohol.

Each $\frac{1}{2}$ c.cm. tinct. digitalis implies 75 mg. of alcohol.

The effect of the two latter preparations was possibly increased by the greater quantity of alcohol involved. In Cat C (pulse tracing in Fig. 2) the injection was 1 c.cm. (1 ampoule) of digalen, undiluted. In the other cases the volume of each injection was 10 c.cm. According to Tereg,¹⁰ the normal secretion of urine in the cat is 200–300 c.cm. per diem, that is, 8 to 12 c.cm. per hour.

In all the above instances sodium sulphate was present in the arterial cannula, and may have accelerated the secretion of urine. But, as its influence would be felt before the injections, as well as after them, it does not affect comparison of the results. A more important consideration is the possible diuretic effect of the normal saline, and of the small quantities of alcohol involved. The influence of these, as well as that of sodium sulphate, is separated from the effect of digalen in the next table.

TABLE III.—Diuretic Effect of Digalen in Comparison with that of its Vehicle.

	Weight of Cat.	Intravenous Dose.	Average Volume of Urine per Hour after Dose.	Duration of Observation.
H	2.2 kg.	20 c.cm. saline 20 c.cm. saline + 0.09 mg. digalen 20 c.cm. saline + 0.18 mg. digalen	10.2 c.cm. 17.4 c.cm. 27.0 c.cm.	50 min. 50 min. 20 min.
K	2.5 kg.	8 c.cm. saline + 56 mg. alcohol 8 c.cm. saline + 56 mg. alcohol + 0.24 mg. digalen	7.1 c.cm. 19.8 c.cm.	60 min. 70 min.

In these two cases no arterial cannula was employed. Sodium sulphate was therefore absent, and the effect of digalen is shown to be considerably in excess of that of its solvent.

None of the above animals was allowed to recover from the anaesthetic. Hence the ultimate effect of the drug on them is not in evidence.

In two cases death followed injection of 0.3 mg. of digalen. In the first, 0.1 mg. (in 3.3 c.cm.) was followed by 0.2 mg. (in 6.6 c.cm.) after an interval of forty minutes, the animal dying half an hour after the second dose. In the second case death took place an hour after injection of 1 c.cm. (1 ampoule). In each case the ventricles were firmly contracted. This dose is, kilo for kilo, about thirty times the therapeutic dose for the human subject.

In no case was haemolysis traceable to digalen injection. This point was further tested *in vitro*, with absolutely negative results, 0.08 mg. of dry digalen dissolved in 3 c.cm. of normal saline, containing cat's corpuscles, producing no trace of haemolysis, whilst one-hundredth as much digitonin liberated an easily recognizable quantity of haemoglobin.

A few observations were made, in the absence of anaesthesia, on rabbits, to test the possibility of local irritation or thrombosis resulting from injections. In one case a rabbit (2 kg.) received, in the course of eight days, hypodermic injections of 1 ampoule (0.3 mg.), $\frac{1}{2}$ ampoule (twice), and $\frac{1}{3}$ ampoule without losing weight or showing any sign of local trouble. In a second case a rabbit (1.6 kg.) was given in a fortnight two intramuscular and two subcutaneous injections of 1 c.cm. (0.3 mg.) each and four intravenous injections of 0.5 c.cm. without any trace of pain, tenderness, or thrombosis. During this period the rabbit gained 25 grams in weight.

Observations on the Frog's Heart.

The hearts of pithed frogs were perfused *in situ*, through the inferior vena cava, from Mariotte flasks containing respectively Ringer's fluid and Ringer's fluid plus pure digalen—that is, without alcohol—in solution, with a perfusion pressure of 2 to 4 cm. After circulating through the frog the fluid escaped from the abdominal veins, which were tied and divided for the purpose. No large artery was cut. The heart was therefore working against vascular resistance, as well as against the weight of the recording lever, which was attached to the apex of the ventricle.

Neave, in the experiments already referred to, perfused the frog's ventricle with digalen solution, diluted 1,000 and 100 times respectively, the latter of which implies 3 parts of dry digalen per million. The first of these solutions had no effect in half an hour, whilst the latter was effective within three minutes.

In the following cases the whole of the heart was exposed to the action of the drug.

Solutions of digalen containing 1 part in 200,000 at first augment the ventricular beat with little or no slowing. Further perfusion with the drug leads to diminution in frequency without immediate reduction in amplitude.

Fig. 4.—Frog heart. Effect of perfusion with digalen, 1 per 100,000. A.—During perfusion with Ringer's fluid, 1 min. before digalen—frequency 34 per min. B.—During fifth minute of perfusion with digalen—frequency 29 per min. C.—During perfusion with Ringer's fluid, 2 min. after digalen, and $2\frac{1}{2}$ min. after B.—frequency 25 per min. D.—During perfusion with Ringer's fluid, 81 min. after a second perfusion with digalen (lasting 6 min.), and 90 min. after C.—frequency 15 per min. The black dot on each tracing shows the level at which ventricular systole starts. The marks below the abscissae indicate seconds. The systolic movement is upward.

Later on the amplitude of the beat may be reduced and the tonus of the ventricle increased.

As with crystalline digitoxin, so also with digalen, the effects of the drug may continue for a time to increase after substituting Ringer's fluid for the drug solution—a phenomenon not noticeable with the less active constituents of the digitalis plant, digitalein and digitonin. With more concentrated solutions of digalen the above sequence of events is more rapid and the effect less readily washed out.

Fig. 4 shows the effect of perfusion with a 1 in 100,000 solution. In this case the frenum cordis was divided; hence the tracings show both the auricular and the ventricular contractions, the former undiminished, the latter reduced by concomitant relaxation of the auricle.

The difference between the records in A and in B was of gradual growth, and the approximation of the heart tracing to the abscissa in the latter appeared, from inspection of the beating heart, to result from increased relaxation during diastole. Towards the end of this perfusion diastolic tonus commenced to increase, but at C was less than at A. Three minutes after C, digalen was again perfused for six to seven minutes, and reduced the frequency of the heart to nineteen per minute. On removal of the digalen the amplitude and the frequency of the beats continued for a while to diminish (the latter to thirteen a minute) and the diastolic tonus to increase, after which amplitude increased and tonus diminished, whilst frequency remained substantially constant (*vide* tracing D). Owing to movement of the apparatus between C and D the tonus in the latter cannot be compared with that in the former tracing.

SUMMARY AND CONCLUSIONS.

In considering the bearing of the above results on the therapeutic applications of digalen to the human subject, it is important to keep in mind the differences between the animals under experiment and the human organism under treatment.

In the former case the drug is producing, for the most part, pressor deviations from the normal state—that is, is working against the resistance of the organism. In the latter case its pressor effects are aided by the natural tendency of the organism towards recovery from depression. Moreover, in the former case the pressor effects are diminished by the anaesthetic. This is exemplified in Table I, where, *caeteris paribus*, the larger effects are seen on the smaller pressures, and the largest of all in Cat 2 after pithing—that is, on the lowest pressures and in the absence of anaesthetic. Hence it may be anticipated that the human subject* in immediate need of digitalis will react to intravenous injections of relatively smaller dose.

The non-production of thrombosis in the small veins of the rabbit seems good evidence that thrombosis would not, with equal care, follow injection into the larger veins of man. It is not, however, fair to conclude that absence of recognizable pain and tenderness from subcutaneous and intramuscular injections in the rabbit implies complete absence of discomfort in man under the same circumstances.

As far as can be judged from the foregoing observations, digalen is an active preparation of digitalis producing characteristic digitalis effects on the circulatory system and on the secretion of urine.

Intravenous, subcutaneous, and intramuscular injections have produced no local trouble.

REFERENCES.

- ¹ Cloetta, *Muench. klin. Woch.*, No. 33, 1904. ² Fraenkel, *Arch. f. exp. Pathol. u. Pharm.*, vol. li, 1904. ³ Cloetta, *Muench. med. Woch.*, No. 47, 1906. ⁴ Cloetta and Fischer, *Arch. f. exp. Pathol. u. Pharm.*, vol. liv, 1906. ⁵ Sasaki, *Berl. klin. Woch.*, No. 26, 1905. ⁶ Neeve, *Scottish Med. and Surg. Journ.*, May, 1907. ⁷ Worth Hale, *Bulletin*, No. 74 of the U.S. Treasury Hygienic Laboratory, Washington, 1911. ⁸ Cushny, *Textbook of Pharmacology*, 1910, p. 372. ⁹ Brunton and Power, *Proc. Roy. Soc. London*, 1874. ¹⁰ Tereg, *Ellenberger's Handbuch d. vgl. Histol. u. Physiol. d. Haussduethiere*, Bd. II, Th. I, p. 342.

* I understand from Dr. Golla that he has, under such circumstances, obtained good results from intravenous injections of 1 c.cm. (= 0.3 mg.) of the preparation.

UNDER the will of the late Mr. Charles Bowles Hare, of Clifton Park, the General Hospital, the Royal Infirmary, and the Eye Hospital, all in Bristol, receive respectively sums of £1,000, £300, and £100.

THE PROTEIN REQUIREMENT; OR, "DO WE EAT TOO MUCH MEAT?"

BY

WILLIAM TIBBLES, M.D., LL.D., L.R.C.P.,
M.R.C.S., ETC.,

MEDICAL OFFICER OF HEALTH, MELTON MOWBRAY RURAL DISTRICT.

VOIT established the rule that the amount of protein required daily was from 1.4 to 1.7 grams per kilo of body weight; or that 119 grams of protein and 2,800 to 3,000 calories of energy were required to meet the demands of the body of a man of average weight when doing light work. Atwater, after many years of work in metabolism, considered that a man—

Without work required 90 grams of protein and 2,700 calories.

With light work required 112 grams of protein and 3,000 calories.

With moderate work required 125 grams of protein and 3,500 calories.

With severe work required 150 grams of protein and 4,500 calories.

Maurel also considers that an adult in health and vigour requires 1.5 grams of protein and 35 to 38 calories of energy per kilo; or 105 grams of protein and 2,450 to 2,660 calories for a man of 154 lb., and 90 grams of protein and 2,100 to 2,280 calories for a woman of 132 lb.

Chittenden, as the result of experiments, believes that 0.1 to 0.12 gram of metabolizable nitrogen, or 0.625 to 0.75 gram of protein, represents the requirements of the body in the ordinary circumstances of life, and that an allowance of 50 to 60 grams of protein daily, or half the amount usually stated in textbooks, is an ample provision for a man of 60 to 70 kilos.

Numerous investigations undertaken by Voit, Moleschott, Ranke, Foster, Munk, Reider, Paton, Zuntz, Lehmann, Pechsel, Klemperer, Hirschfield, and others, show that when no food is taken for twenty-four hours the amount of nitrogen excreted varies from 7.5 to 12 grams, the variation probably being due to a difference in the amount of stored and circulating protein and glycogen in the organism. But if the starvation is continued the stored protein and glycogen is all consumed in a few days, and after a period of about five days all the nitrogen in the excretions will be derived from the disintegration of tissue proteins. In fasting people the amount of nitrogen excreted by the kidneys from the fifth day of starvation averages about 4.5 grams per day. If we allow a margin of 1 gram for other modes of excretion, for example, the skin and mucous surfaces, we arrive at the conclusion that the amount of protein absolutely essential to prevent the destruction of tissue proteins is $N\ 5.5 \times 6.25 = 34$ grams daily, or about 0.5 gram of protein per kilo of body weight. *The physiological minimum for an adult is therefore 34 grams of protein daily.* But other points have to be considered. The observations of Chittenden, Hirschfield, Klemperer, Siven, Pechsel, and others, have clearly demonstrated that it is possible to maintain life and keep the body in nitrogen equilibrium, and even do a certain amount of work, with a diet having the standard energy value, but containing a very much smaller amount of protein than is usually considered necessary. It remains to be seen how far the protein requirement is influenced by work. It is possible for protein to supply all the energy required by the body, as well as to maintain the body in nutritive and nitrogenous equilibrium. But whether it is necessary or even advantageous for the body to derive part of its energy from protein is another matter. There are people who perforce live almost exclusively on animal food for six or nine months of each year. In such cases the energy of the body is derived from the fatty moiety of the protein molecule or from the carbohydrate complex which exists in many proteins. But what we want to know is whether work increases the demand for protein food. Pflüger advanced the theory that protein is the source of all muscular energy. Against this theory, no longer held, is the fact that no evidence has been adduced which clearly shows there is any change in the condition of the muscle proteins when muscle contracts. Hermann's theory of muscular contraction assumes that there is a change in the muscular proteins similar to what occurs in rigor mortis. But nobody has actually observed the formation