As to the geological age, the evidence obtained goes far to prove that this "sub-fossil" fauna existed at a comparatively very recent period, and that Man was contemporary with these Vertebrates. The evidence of various kinds may be arranged as follows:—

1. The very fresh aspect of all the remains, which are
2. Found in marshes and in recent alluvia (dunes), the formation of which is still in progress.
3. *Crocodilus robustus* is still existing in lakes in the interior.
4. Some of the bones bear traces of Man's handiwork.
5. The record of a monstrous animal, probably the *Hippopotamus*, is preserved in the legends of the natives.
6. Amongst the accounts—brief descriptions with native names—given by a trustworthy explorer of the seventeenth century, De Flacourt, are several which, on account of size and other characters assigned to them, cannot be identified with any animals actually existing in the island. One of the descriptions may possibly refer to *Megaladapis* and that of a bird, from the size of an Ostrich to one of the species of *Æpyornis*.
7. Remains of domestic cattle were found, together with the bones of the extinct forms of Mammals, &c.

On the other hand, evidence is adduced in support of the almost certainty that a *Tertiary* Vertebrate fauna will be, sooner or later, forthcoming in Madagascar, owing to the recent discovery of Tertiary lacustrine deposits in several different localities in that island.

XVII. "Some of the Effects and Chemical Changes of Sugar injected into a Vein." By Vaughan Harley, M.D., Teacher of Chemical Pathology, University College, London, Grocer Research Scholar. Communicated by George Harley, M.D., F.R.S. Received June 13, 1893.

(From the Physiological Institute, Leipzig.)

It being no longer doubted that sugar is essential to life, and it having been experimentally shown that the animal organism can form saccharine matter out of proteids, as well as carbohydrates, it becomes of importance to know what changes sugar undergoes in the body before its elements are finally eliminated.

According to present knowledge, it is considered that by the three chemical processes of hydration, oxidation, and reduction, the molecule of sugar is changed into larger and smaller molecular groups, and that it is in the breaking down and building up of these that heat and vital energy are developed. The basis of this belief rests on the observation that when sugar is artificially introduced into the

system its disappearance is accompanied by the liberation of heat and the development of muscular energy.

To discover the *modus operandi* of this is a matter of extreme difficulty, from the fact that when sugar is artificially introduced into the living circulation it is rapidly distributed to every organ of the body. In each of these it undergoes specific changes. So that, while the number of its derivatives are great, the amount of each is proportionally small. So small is it, indeed, of some of them, as to render their collection in sufficient quantity for the purposes of investigation next to impossible. However, by the advice and kind assistance of Professor C. Ludwig, I made the attempt, and the present communication, which I have the honour to lay before the Royal Society, embodies in brief the results of the research.

The methods adopted were briefly as follows:—As sugar artificially introduced into the veins is rapidly, and in different animals in varying proportions, eliminated by the kidneys, the ureters were ligatured so as to retain the sugar in the organism. In all cases where it was intended to kill the dogs within a few hours, the ligaturing was done from the front. Whereas, when it was intended to keep the dog alive, the operation was performed from behind, and the ureters tied by means of a ligature-staff, so that, at any given time, the ligatures could be removed without re-opening the wound. In order that all the animals should be under exactly the same conditions, in so far as their assimilative functions were concerned, no food was given to them during the previous twenty-four hours.

A 50 per cent. chemically pure grape sugar, dissolved in normal saline, was slowly introduced into the jugular vein. 10 grams of sugar to every kilo. of the dog's weight were the most usual quantities employed, and the whole introduced in about an hour.

After the necessary treatment of the various organs, the quantity of sugar was estimated by Allihn's method, the quantity of lactic acid by Drechsel's method, and the quantity of glycogen by Kulz's method. Alcohol, acetone, acetoacetic acid, β-oxybutyric acid, and ammonia were tested for in the usual way.

The following is a summary of the facts obtained:—

1. *Nervous Phenomena.*—It was noticed that large quantities of sugar gave rise, in dogs, to a series of symptoms pointing to nerve centre poisoning, their severity varying in different cases.

These nerve symptoms were generally preceded by, or attended with efforts at vomiting. But, from their stomachs being empty, the animals only brought up frothy mucus.

In some cases, the irritation of the nerve centres was only shown by muscular movements, causing a trembling of the skin. In other instances the muscular contractions were sufficiently severe to cause a quivering of the limbs, or even well-marked convulsions.

During the convulsive stage the pupils were contracted, although still reacting to light. The respirations were greatly increased. Around the mouth frothy mucus collects. The convulsions alternated with periods of rest, which gradually prolonged themselves until the dog seemed to be asleep. The convulsions sometimes were followed by profound coma, out of which the animal could not be roused. In some of the dogs the coma set in without any preliminary convulsive stage. Both the convulsive and comatose state always passed off when the ureters were loosed, while in some instances all the symptoms disappeared of their own accord.

10 grams of sugar per kilo. usually proved fatal to small dogs during the convulsion stage, from the respirations suddenly ceasing. Large dogs (of 20, or more, kilos. weight), showed only very mild symptoms, even after as much as 12 grams of sugar per kilo. weight were employed.

From the order of appearance and disappearance of the symptoms above alluded to, it seemed as if the sugar, by the breaking up of its molecule, yielded a poison or poisons, which, on being further transformed, became harmless.

It is worthy of note that none of these symptoms occur when sugar is absorbed from the intestines, no doubt for the same reason as they do not occur when only small quantities of sugar are injected into a vein; namely, because the intermediate products are never present at any one time in sufficient quantity to produce them.

2. *The quantity of sugar that remained as sugar in the different tissues* of the body was estimated at various periods of time after the completion of its injection.

(*a.*) The *quantity of sugar in the blood* at different periods of time after its injection into the jugular vein, the ureters being ligatured.

No. of experiment.	Time after completion of sugar injection.	Sugar in 100 parts of blood.
IX	Before injection	?
	1 hour after	?
	6 hours after	0·095
X	Before injection	0·112
	1 hour after	0·438
	3 hours after	0·126
	6 hours after	0·084
XI	Before injection	0·079
	Immediately after	0·676
	4 hours after	0·311
	6 hours after	0·118

From this it was seen that the quantity of sugar goes on diminishing after its injection, and that by the sixth hour it had reached (Exp. X) even a lower point than what it stood at after the twenty-four hours' fast.

(*b.*) *The quantity of sugar found in the liver*, as compared with that met with in the blood.

No. of experiment.	Time after completion of sugar injection.	Sugar in 100 parts of	
		Moist liver.	Blood.
IV	6 h. 20 m.	0·92	0·056
III	7 h.	1·72	0·025

The great difference observable between the quantity of sugar in the liver and in the blood shows how imperfect must be the passage from the liver into the general circulation of such a diffusible substance as sugar. This is all the more remarkable when we remember that the liver cells are surrounded not only by blood, but also by lymph, vessels.

(*c.*) *The Presence of Sugar in the matter vomited.*—As previously mentioned, several of the dogs vomited a quantity of mucus after the injection of the sugar.

In the cases in which the vomit was analysed it was found to contain sugar in small quantities.

(*d.*) *Quantity of Sugar in the Alimentary Tract.*—In order to ascertain if any of the sugar was eliminated into the alimentary tract, the contents of the stomach and intestines of three dogs were analysed. As all were in a fasting condition, contamination from food had not to be feared.

In only one case was sugar found in the stomach, and then only to the amount of 0·174 gramme. In no case was it found in the intestines. So it had, in all probability, been eliminated by the salivary glands and swallowed with the saliva. I am led to this opinion on account of Weyert having pointed out that when large quantities of sugar are injected into a vein, it is, in small quantities, eliminated with the saliva.

One may therefore, I think, consider that the alimentary tract does not eliminate sugar.

(*e.*) *Quantity of sugar in the œdematous exudations*, collected from the kidneys and upper part of ureters after their ligature.

No. of experiment.	Time after completion of sugar injection.	Quantity of fluid.	Quantity of sugar.	Sugar in 100 parts of	
				Œdematous fluid.	Blood.
		c.c.			
V	1 h. 45 m.	16	0·463	2·89	0·290
II	7 h.	4	0·104	2·60	0·064
I	7 h.	10	0·196	1·964	—
III	7 h.	11	0·105	0·96	0·025

Here it is seen that the quantity of sugar in the œdematous fluid, as was observed in the case of the liver, is greater than in the blood.

(*f.*) *Quantity of Sugar in the Urine.*—In order to ascertain if sugar passed out in the urine six or more hours after its injection, the ligatures were removed from the ureters in five of the cases and the urine passed during the following night collected. In one case only was there as much as 0·06 per cent. of sugar found in the urine. Consequently one may conclude that after sugar is injected and the ureters kept ligatured six or more hours, no sugar is eliminated in the form of sugar by the kidneys. The small quantity found in the one case could have been derived from the exudation which had accumulated in the kidneys and ureters during the time of ligature.

3. *Quantity of glycogen found in the liver and muscles* after the injection of sugar into the jugular vein.

No. of experiment.	Time after completion of sugar injection.	Glycogen in 100 parts of	
		Liver.	Muscle.
V	1 h. 45 m.	0·43	0·22
IV	6 h. 20 m.	0·52	0·39
I	7 h.	2·31	0·81
II	7 h.	1·75	0·09
III	7 h.	9·65	—

The results obtained from these five experiments show that the quantity of glycogen met with after the injection of 10 grammes of sugar per kilo. of dog's weight is well within the ordinary limits. Consequently one cannot say that any very appreciable amount of glycogen had been formed from the sugar that had been artificially introduced into the general circulation.

4. As it was thought possible that *lactic acid* might be one of the substances into which sugar split up in the organism, quantitative estimations were made of it in the blood and certain of the tissues.

(*a.*) *Quantity of lactic acid in the blood* at various times after the intravenous injection of sugar.

No. of experiment.	Time after completion of sugar injection.	Lactic acid in 100 parts of blood.
IX	Before injection	0·106
	1 hour after	0·112
	6 hours after	0·069
X	Before injection	0·071
	1 hour after	0·107
	3 hours after	0·104
	6 hours after	0·084
XI	Before injection	0·097
	Immediately after	0·134
	4 hours after	0·116
	6 hours after	0·110

It is here seen that a marked increase of the lactic acid takes place after the injection of sugar. Further, that after the fourth hour it decreases slower than does the percentage of sugar itself in the blood. In the following table the effects of this slower decrease is well shown.

No. of experiment..	XV.	XVI.	IV.	III.	VI.
Time after injection	4 hours.	5 hours.	6 hours.	7 hours.	25 hours.
Sugar per cent.	0·072	0·073	0·056	0·025	0·090
Lactic acid per cent.	0·087	0·082	0·090	0·090	0·130

These results give an average of 0·065 per cent. of sugar as against 0·096 per cent. of lactic acid in the blood. Whereas, in dogs on normal diet, Gaglio has shown the quantity of sugar in the blood was more than the quantity of lactic acid.

(*b.*) *Quantity of lactic acid in the liver and muscles* as compared with the amount in the blood after ligature of the ureters and the injection of 10 grammes of sugar per kilo. of dog's weight.

No. of experiment.	Time after completion of sugar injection.	Lactic acid in 100 parts of		
		Liver.	Muscle.	Blood.
V	1 h. 45 m.	0·339	0·171	0·135
IV	6 h. 20 m.	0·135	0·112	0·090
III	7 h.	0·031	0·011	0·090

The liver is thus seen to contain more lactic acid than the muscles, while, on the other hand, the blood sometimes contains more and sometimes less than both the liver and muscles.

From this it might be inferred that if the amount of a substance in any given part of the body yields a clue to the seat of its formation, it is probable that the liver is the principal seat of the lactic acid formation, as pointed out by Wyssokowitzsch.

(*c.*) *Quantity of lactic acid excreted in the urine*, the ureters having been ligatured for six or more hours after the injection of sugar into the jugular vein.

No. of experiment.	Ureters having been ligatured.	Time during which the urine was collected.	Quantity of urine.	Contained lactic acid.	
				Total quantity in grammes.	Per cent.
			c.c.		
XV	6 h.	First 12 hours	475	0·115	0·024
		Following 24 hours	600	0·062	0·010
VIII	6 h.	„ 12 „	230	0·173	0·075
XI	6 h. 40 m.	„ 24 „	650	0·571	0·088
V	9 h.	„ 12 „	162	0·562	0·346

From these results it appears that lactic acid is not only increased in the blood after the injection of sugar, but that it is at the same time excreted in the urine, whereas lactic acid is not known to occur in healthy dog's urine.

5. As *acetone, acetoacetic acid, and β-oxybutyric acid* are often found *in the urine* of diabetics, it was decided to examine it for these substances.

(*a.*) *Acetone and acetoacetic acid* were found to be present in all the five urines examined.

(*b.*) *β-Oxybutyric acid* was searched for in the urine both with the

polariscope and as α-crotonic acid, but could not, with any certainty, be demonstrated.

6. The *blood* was examined for *alcohol, acetone, and acetoacetic acid* by distilling it at a temperature of 102° C. by means of steam passed through it. The distillate being examined for alcohol and acetone. While in normal blood so treated neither alcohol nor acetone could be demonstrated, in the blood of dogs subjected to the sugar injection the presence of both of these substances was readily recognised.

Acetoacetic acid was searched for by acidifying the blood with sulphuric acid and then again distilling it. By this means its presence was also demonstrated in the blood of the sugared dogs.

7. As not only alcohol and acetone but likewise acetoacetic acid was found in the blood after the intravenous injection of sugar, it was decided to see how much *ammonia* could also be distilled off after adding magnesia to the blood. In the first place, as it was necessary to see if ligaturing the ureters caused an increase of the ammonia normally present in the blood. The blood of a dog in which the ureters alone had been ligatured was distilled, first immediately after and then a second time when the ureters had been ligatured five hours. The first blood yielded 0·025 per cent. and the second 0·024 per cent. of ammonia. So the ligaturing of the ureters had caused no increase in the quantity of ammonia present in the blood.

The same thing was then done with the blood from dogs before and after the intravenous injection of sugar.

Weight of dog.	Ammonia obtained from 100 parts of blood.	
	Before the sugar injection.	5 hours after.
kilos.		
29·5	0·025	0·034
27·5	0·018	0·017
5·0	0·042	—
3·8	0·039	—

It is thus seen the sugar injection had caused no increase in the quantity of ammonia in the blood.

It may be safely concluded that ammonia is not the cause of the nervous symptoms which followed the injection of 1 per cent. of sugar intravenously.

As these results again do not accord with those which follow on the injection of alcohol, we are led to suppose that they may be in some way or another connected with the presence in the blood of the abnormal substances, acetone and acetoacetic acid, which, as was seen, were not only found in the blood but likewise in the urine.

The possibility that the increase in the quantity of the lactic acid may likewise assist, if not be a material cause, in produciug the nerve-symptoms, is also worthy of note.

That sugar, acting as sugar, was not their cause was shown by the symptoms not appearing until from a quarter to one hour after its injection. That is to say, not till the quantity of sugar present in the blood had already markedly decreased.

XVIII. "Experiments on Variola and Vaccinia." By S. Monckton Copeman, M.A., M.D. (Cantab.). Communicated by Professor M. Foster, Sec. R.S. Received June 14, 1893.

In the course of some experiments on the bacteriology of vaccine lymph, I was confronted by a difficulty in the practical testing of certain of the results of my work. Continuous experimentation on children being obviously out of the question, I was naturally led to turn my attention to the discovery if possible, of some one or more of the lower animals which, by reason of their passing through the various stages of vaccination and more especially variolation in a manner comparable with that witnessed in the human subject, might serve me for control experiments.

Little or no success having been obtained with the various domestic animals, I next turned to the monkey tribe on account of their similarity in many respects to man, although assured at the time that they were not susceptible to either vaccinia or variola. On putting the matter to the test, however, I found that this was not the case, the inoculation of vaccine and of variolous lymph having each of them given, in my hands, successful results in every instance in which I have tried it on the monkey (*Rhæsus*).

In the case alike of variola and of vaccinia, the local result of inoculation attains its acme (*quâ* vesiculation) in the monkey, as in the human being, about the eighth day. The first signs of reaction appear usually on the third day, by which time, if variolous lymph has been used, there is a distinct, though very thin, crust over the site of inoculation. By the fifth day vesiculation has generally commenced, this becoming more obvious up to the eighth day, though even then it is much less marked in variolous cases than in those which have been vaccinated, the difference being easily recognisable.

Later the vesicle gives rise to a pustule, by which time there is not infrequently considerable swelling of the skin and subcutaneous tissue and of the nearest lymphatic glands. The pustule gradually dries up, and a scab is formed which is more pronounced after vaccination than variolation, and which falls off some time during the third week, if the monkey has not picked it off before.