

and there results an increase in the free cholesterol of the blood. Phytosterol does not appear in the blood as such.

We take this opportunity of expressing our thanks to the Government Grant Committee of the Royal Society for assistance in carrying out this work.

On the Comparative Toxicity of Theobromine and Caffeine, as Measured by their Direct Effect upon the Contractility of Isolated Muscle.

By V. H. VELEY, F.R.S., and A. D. WALLER, M.D., F.R.S.

(Received June 17,—Read June 30, 1910.)

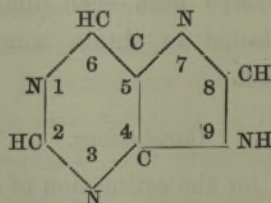
Introductory.

The isolation of caffeine from coffee is generally assigned to Robiquet, Pelletier and Caventou (1821),* though the so-called coffee base, obtained a year previously by Runge† was probably impure caffeine.

The identity of theine, extracted from tea by Oudey (1838),‡ with caffeine was established by the analyses of Jobst (1843).§ The association of caffeine with uric acid was noted originally by Stenhouse,|| who found the latter in a sample of Paraguay tea.

Theobromine was first isolated by Woskrcsensky¶ from cocoa (1842), and its association with alloxan, on the one hand, and caffeine on the other, was established by subsequent observers.

According to the investigations of Emil Fischer (1894—1898),** uric acid, theobromine, caffeine, and other allied substances, are derived from the parent base purine, to which the following, as one of two alternative formulæ, was eventually assigned:—



* 'Berzelius Jahresber.', vol. 4, p. 180, and vol. 7, p. 269.

† 'Phytochemischer Entdeckungen,' p. 144, Berlin, 1820.

‡ 'Mag. Pharm.,' vol. 19, p. 49.

§ 'Annalen,' 1838, vol. 35, p. 63.

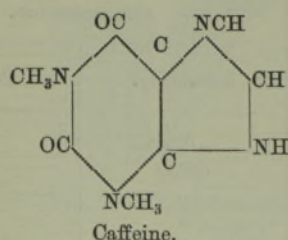
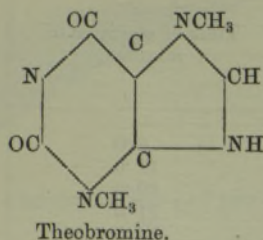
|| *Ibid.*, 1843, vol. 45, p. 371, and vol. 46, p. 227.

¶ *Ibid.*, 1842, vol. 41, p. 125.

** 'Untersuchungen in der Purin-Gruppe.' Collected volume, Berlin, 1907.

The figures denote the relative positions of the substituting groups proposed originally by Fischer, and now adopted in chemical literature.

Theobromine is 3,7-dimethyl-2,6-dioxypurine, and caffeine or theine is 1,3,7-trimethyl-2,6-dioxypurine:—



Hypoxanthine is monoxypurine and guanine its mono-amino derivative, xanthine is dioxypurine and uric acid is trioxypurine.

Unfortunately, the bases guanine and xanthine, as also uric acid, are too insoluble in water (less than 1 part in 1000) for the purpose of our investigation. Among coffee extractives is caffee-tannic acid, $C_{15}H_{18}O_2$, which occurs as a magnesium or calcium salt in the berries (Rochleder)*; it is a gummy substance readily soluble in water and alcohol.

The substances investigated are theobromine, caffeine, caffee-tannic acid, and an artificial mixture of caffeine and tannic acid, as also extracts of tea and coffee (with or without caffeine).

Caffeine (Trimethyldioxypurine).

All the records obtained were similar in type, the muscle going into contracture, and abolition finally taking place in a longer or shorter time according to the concentration.

In the record given (fig. 1), which is quite characteristic, a $n/200$ solution of caffeine (0.1 per cent.) produced abolition in 11.5 minutes; in other experiments on other days with solutions of the same concentration the times were 10.5 and 11 minutes respectively, and in a fourth experiment on a cooler day, and consequently at a lower temperature (which, however, we omitted to note), the time was 14 minutes. With a solution of double the concentration, namely, $n/100$ (0.2 per cent.), abolition took place in 4 minutes.

In earlier experiments with caffeine hydrochloride and hydrobromide solutions ($n/200$ concentration) contracture took place immediately; this more rapid effect is attributable to the conjoint action of the caffeine and the

* 'Annalen,' vol. 59, p. 300.

acid, as these salts under these conditions are nearly completely hydrolysed into their acid and basic constituents.* The above results are summarised below :—

Caffeine.

Concentration.		Time of abolition in minutes.
Normality.	Per cent.	
$n/100$	0·2	4·0
$n/200$	0·1	11·5
"	"	10·5
"	"	11·0
"	"	14·0

Caffeotannic Acid.

A sample of this substance (Merck), a brown gummy material, as previously described, was made up to $n/100$ concentration (0·32 per cent.) in normal saline, but was found to be practically inactive. A solution of double the strength ($n/50$ or 0·65 per cent.) produced abolition in 21 minutes, the record resembling that of caffeine. By way of comparison a solution was made up of $n/100$ (= 0·2 per cent.) caffeine and 0·5 per cent. of a sample of tannic acid, presumably obtained from oak-bark; the time required for abolition was 20 minutes, namely, five times longer than that for caffeine of the same concentration by itself. The record can be characterised as a retarded caffeine record, suggesting that the free tannic acid added to the caffeine causes some secondary or retarding chemical change.

Theobromine (Dimethyldioxyypurine).

All the records obtained with this base were similar in type, and resembled those of caffeine; in the record given (fig. 2) for a theobromine solution of $n/200$ (or 0·09 per cent.) concentration, abolition took place in 6·5 minutes. The results obtained are summarised as under :—

Theobromine.

Concentration.		Time of abolition in minutes.
Normality.	Per cent.	
$n/150$	0·12	2·5
$n/200$	0·09	6·5
$n/300$	0·06	8·5
"	0·06	9·0

* Schmidt, 'Annalen,' 1883, vol. 217, p. 284; Veley, 'Journ. Chem. Soc. Trans.,' 1909, vol. 93, p. 664.

The concentrations are in the ratio 1:1.5:2 and the times are in the ratio 3:2:1, with an error of 0.5 minute, which is that of experiment.

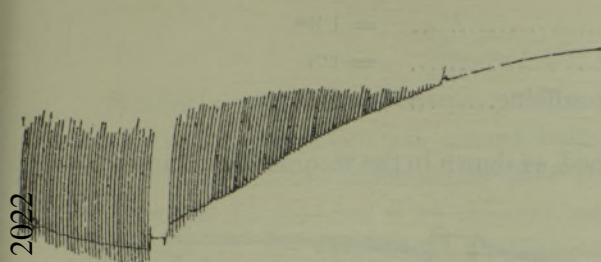


FIG. 1.—Caffeine, $n/200$ (0.1 per cent.).

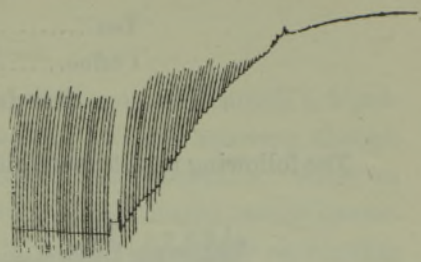


FIG. 2.—Theobromine, $n/200$ (0.09 per cent.).

On comparing theobromine and caffeine solutions of like concentration, namely, $n/200$ (which was actually done simultaneously with a pair of muscles), the times required for abolition are in the ratio 6.5:11 or 1:1.7; again, on comparing a $n/300$ solution of theobromine with a $n/200$ solution of caffeine the times are in the ratio 9:11 or 1:1.2; which would be nearly equivalent to a ratio 1:1.8 in terms of the same concentration. From these results we conclude that the toxic value of theobromine is to that of caffeine approximately as 1.7:1 for equal molecules or 1.8:1 for equal weights. It appears that the introduction of the methyl group into theobromine to form caffeine lowers the toxic value, a result which is the converse of that obtained in the case of the paraffinoid alcohols* and unlike those of methyl stovaine and stovaine,† and of pyridine and picoline,‡ in which such introduction of a methyl group produced approximately no alteration of activity.

Coffee and Tea Extracts.

We also made a few observations with ordinary extracts of coffee, with and without caffeine, and of tea; herein we are indebted to the Life Belt Coffee Company, Ltd., for supplying us with samples of "coffee freed from caffeine"; it appears from the analyses given that by the process adopted 90 per cent., or thereabouts, of the caffeine is removed. For the purposes of these experiments we made our "tea" and "coffee" by boiling in physiological saline solution in the proportion of 2.5 and 5 grammes per 100 c.c., filtering off the solid matter and using the extracts. In order to determine the amount of solid dissolved under such conditions 50 c.c. of the 5 per cent. extracts were evaporated to dryness and the residue weighed. After deduction of the

* Kemp, 'Journ. Physiol.,' 1908, vol. 37, p. 43.

† Veley and Waller, 'Proc. Roy. Soc.,' B, 1910, vol. 82, p. 147.

‡ *Ibid.*, p. 336.

weight of the salt in the physiological saline solution, the net weights of the residues (grammes per 100 c.c.) were found to be as under:—

Tea.....	= 1.98
Coffee.....	= 1.8
„ free from caffeine.....	= 1.48

The following results were obtained, as shown in the records (figs. 3 and 4):—

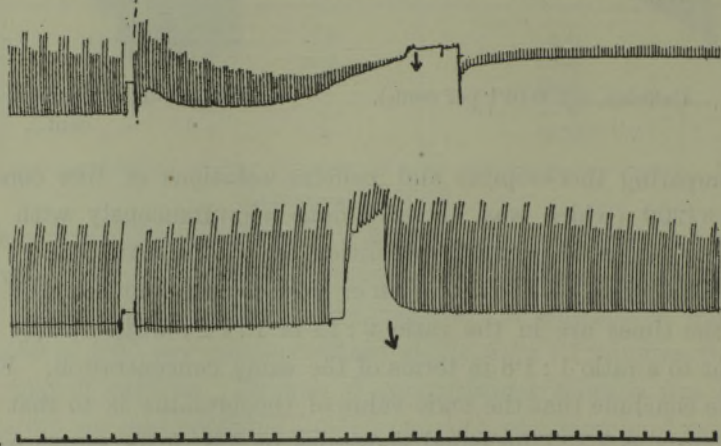


FIG. 3.—Saline Tea. Upper line 2.5 per cent. Lower line 1 per cent., then 5 per cent. (At the points marked by arrows, simple saline was substituted for the saline tea.)

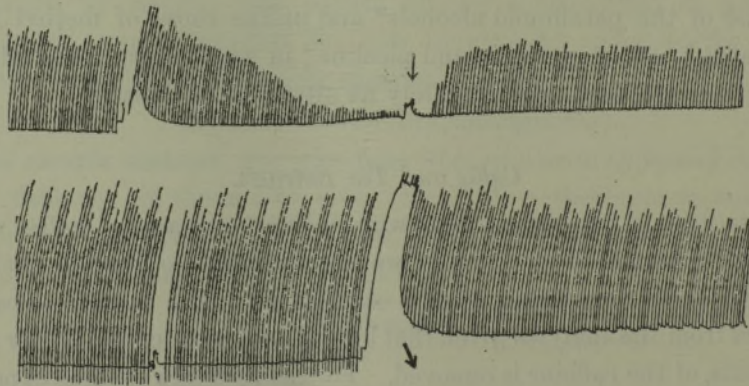


FIG. 4.—Saline Coffee. Upper line 2.5 per cent. Lower line 1 per cent., then 5 per cent. (At the points marked by arrows, simple saline was substituted for the saline coffee.)

Saline Tea Extract.

A 1-per-cent. extract produced no effect; on substituting a 5-per-cent. extract, there was an immediate contracture followed by an equally immediate recovery on replacement of saline solution. After an interval of 19 minutes a 2.5-per-cent. extract was substituted, which caused a slight contracture,

abolition taking place in 12·5 minutes, followed by a slight recovery on reintroducing saline solution.

Saline Coffee Extract.

Similarly a 1-per-cent. extract produced no effect; on substituting a 5-per-cent. extract, the effect was almost instantaneous, and the recovery, though incomplete, followed immediately after running in saline solution. After an interval of 19 minutes a 2·5-per-cent. extract was substituted, which caused a contracture with abolition in 12·5 minutes, followed by recovery on running in saline solution.

There do not appear to be any very marked differences between the saline tea and coffee extracts; though possibly the contracture is more marked in the case of the latter, the recovery is less marked in the case of the former. Such a result would be probable, if the toxic effect both of tea and of coffee is mainly due to the caffeine (theine) present, the tannic acid, also a constituent of both, producing only a secondary effect as regards muscle proteins.

But with the extract of the "caffeine-free coffee" (fig. 5) there was

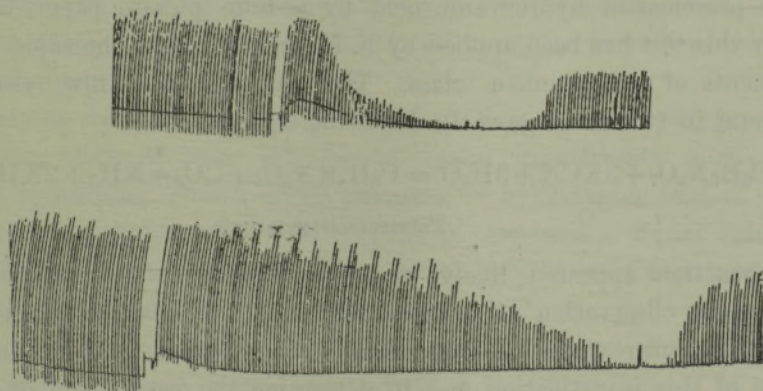


FIG. 5.—Saline Coffee "freed from caffeine." Upper line 5 per cent. Lower line 2·5 per cent.

no such marked contracture on adding a 5-per-cent. extract, and abolition took place in (nearly) nine minutes; in the case of a 2·5 extract, abolition took place in 21 minutes, followed by recovery on substituting saline solution. The differences between the effects produced by ordinary and "caffeine-free" coffee are most marked, though in the case of the 2·5-per-cent. extracts they would doubtless have been less pronounced if the experiments had been both equally performed with a fresh muscle.

But, as stated above, the experiments, though striking in their results, were rather of a domestic character than of physical precision. However this may be, the difference between ordinary and "caffeine-free" coffee affords a further proof that the toxic effect is principally due to the presence of the caffeine.

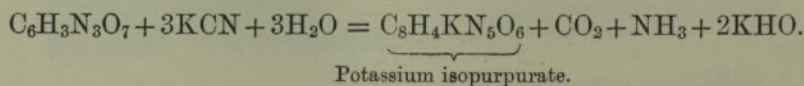
A New Method for the Quantitative Estimation of Hydrocyanic Acid in Vegetable and Animal Tissues.

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(Received June 17,—Read June 30, 1910.)

(From the Physiological Laboratory of the University of London, South Kensington.)

The evolution of hydrocyanic acid by laurel leaves (*Prunus laurocerasus*) in consequence of congelation, or of their exposure to the action of anæsthetic vapours, was first pointed out by Raphael Dubois.* It has been studied more recently by Guignard,† who has introduced an extremely delicate test for the presence of hydrocyanic acid by sodium picrate paper, and quite recently this test has been applied by F. E. Armstrong‡ for the rapid detection of ferments of the emulsin class. The reaction was first studied by Hlasiwetz§ in 1859, who gave the following equation:—



The reaction appeared likely to afford a convenient instance for the simultaneous observation of chemical and electrical changes taking place in living protoplasm under the influence of anæsthetics, and the immediate purpose of this investigation was to determine the parallelism or the want of parallelism between the course of the two changes—chemical and electrical.

Qualitative experiments in which the evolution of hydrocyanic acid from laurel leaves was followed by means of picrate paper, the leaves being enclosed in corked tubes containing the vapour of (1) chloroform; (2) ether; (3) alcohol; and (4) water, gave results in the order of intensity (1), (2), (3),

* R. Dubois, 'Richet's Dictionnaire de Physiologie,' art. "Hydratation."

† Guignard, 'Bulletin des Sciences Pharmacologiques,' 1906, p. 415.

‡ E. F. Armstrong, 'Physiol. Soc. Proc.,' March 19, 1910.

§ Hlasiwetz, Liebig's 'Annalen,' vol. 110, p. 289, 1859.