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Chemical Blood Studies.*

IV. Comparative Studies on "Laked" and "Unlaked" Blood Filtrates of Horses in Health and during Horse-sickness (*Pestis equorum*).

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CONTENTS.

I.—INTRODUCTORY NOTE.

The present paper is one of a series of similar researches into various diseases of animals. In order to avoid needless repetition, the aims and objects of these studies, the chemical methods and technique employed and the arrangement of the data have been collected and fully discussed in the first paper (see under "References" for a list of articles published up to now in this series).

II.—HORSE-SICKNESS (*Pestis equorum*).

(*a*) SYMPTOMATOLOGY AND PATHOLOGICAL ANATOMY.

Although it is not intended to here give a full description of this disease, it may be of interest briefly to state the symptoms and post-mortem findings, more particularly with a view to correlating abnormal blood conditions, if any, with the symptomatology or the pathological anatomical diagnosis.

For fuller details the bibliography at the end of this article should be consulted. No reference in the available literature to any purely biochemical researches with horse-sickness blood could be found; physico-chemical by Frei (1907) and blood morphological researches by Neser (1923 and 1926) constitute the only investigations into the blood of equines during an attack of horse-sickness.

* "Chemical Blood Studies, I, III–V" was accepted as Thesis for the D.V.Sc. degree by the University of Pretoria, December, 1932.

Horse-sickness has been defined (Knuth and du Toit, 1921, and Theiler, 1921) as " an acute and sub-acute infectious disease of equines, caused by an ultravisible virus, and which is generally fatal. It is not directly contagious, infection most likely being spread by bloodsucking insects. Animals which have recovered are immune and no longer harbour the virus. It occurs in South and Central Africa."

The disease is readily produced in a susceptible animal by the sub-inoculation of blood or serum from a reacting animal.

Clinically four different forms are distinguished, viz., horse-sickness fever, the pulmonary form (Dunkop), the oedematous or cardiac form (Dikkop), and the " mixed " form.

(*a*) *Horse-sickness Fever* is primarily characterised by its typical intermittent temperature reaction which sets in usually 5–7 days after infection, reaching its acme by the 9th to 13th day p.i., the hyperthermia decreasing generally by lysis. Other symptoms, except usually a slight loss of appetite and dullness, are absent.

(*b*) The course of the *pulmonary form* is usually fulminant or peracute, with a short incubation period of 2–5 days, rarely longer. The temperature rises rapidly to 105–106°, the animals frequently succumbing at the acme of the reaction. The characteristic symptoms noted are (in addition to hyperrhexia) a very severe dyspnoea of both inspiratory and expiratory character; coughing which is as a rule followed by a discharge of often large amounts of a yellow frothy liquid, great restlessness, dilatation of the pupils, cyanosis of the mucous membranes and death. In cases of recovery, the symptoms are less acute and the temperature reaction shows a more prolonged stadium incrementi and decrementi.

(*c*) The *oedematous* or *cardiac form* (Dikkop) is characterised by (i) an incubation period of 5–7 days, the acme being reached usually on the 12–13th day p.i., succeeded by a critic or lytic descent; the duration of the fever lasting generally 8–10 days. (ii) The development of subcutaneous oedematous swellings, particularly of the head and neck, shortly after the fever acme has been passed. These swellings are tense to the touch, as a rule not painful and in cases of recovery disappear in a few days. (iii) Cardiac symptoms as evinced by the cyanotic mucous membranes, alterations in the quality and rate of the pulse, which increases to 50 to 60, becoming gradually weaker, softer and thready; arythmic, dicrotic or deficient pulses are also met with. The cardiac area of impulse increases, the heart sounds becoming weaker and more diffuse. A cooling of the extremities is often noted, especially in fatal cases. (iv) A dyspnoea, which, however, is not as pronounced as in the Dunkop form. (v) Loss of appetite setting in at the onset of the fever reaction and going over to complete anorexia is often seen. Complications such as paralysis of the oesophagus, gangrenous pneumonia and colic may be sometimes observed.

Pathological Anatomy.

At post-mortem the following characteristic changes are observed:—

(*a*) The pulmonary (Dunkop) form shows a severe oedematous transudation into the tissues of the lungs, subpleural tissue, regional lymph glands and submucosa of the trachea with a clear greenish-yellow fluid; hydrothorax, the pleural cavity containing up to several litres of clear fluid; subepicardial and subendocardial haemorrhages; hyperaemia of the liver and kidneys—rarely associated with degenerative changes.

(*b*) The cardiac, oedematous or "Dikkop" form is characterised by transudation of the subcutical tissues with a pale yellowish fluid chiefly in the region of the head and neck, fasciae and muscular aponeuroses and regional lymph glands; cyanosis of the mucous membranes; hydropericardium—the amount of liquid varying from $\frac{1}{2}$-litre to $2\frac{1}{2}$-litres; extensive subepicardial and subendocardial haemorrhages; hyperaemia and fatty degeneration of liver and kidneys, degeneration of the myocardium.

Experiments with Horse-sickness.

For these analyses blood was drawn from horses which had been artificially infected by inoculation with virulent blood (except Case III) for investigations also into other aspects of the disease, particularly transmission and immunisation experiments. I here wish to place on record my sincere thanks to my colleagues Messrs. W. O. Neitz and R. du Toit, for their ready permission to bleed their experimental subjects at any time for the purpose of my analyses and for their courtesy in informing me in good time of likely suitable cases.

The horse were of various breeds and conditions, and ages, details being given under "History" in each case. The rations supplied are given in "Chemical Blood Studies" I (see this Journal).

Most of the animals were placed on temperature for months or weeks prior to infection, but in this paper only the temperature records of the last 2–3 weeks have been reproduced. The normal temperature records run a remarkably even course, showing for months on end only the slight diurnal variations, with a "mean" level at about 100°.

(*b*) Methods of Analysis and Technique.

Exactly the same procedure was followed as that described in the first paper of this series ("Chemical Blood Studies I," this Journal). In connexion with the study of equine blood, particularly horse blood, the precaution of *thorough* shaking of the oxalated blood must be observed before pipetting off any blood for the preparation of filtrates, total nitrogen and haemoglobin determinations, otherwise the results will be inaccurate, because with equine blood sedimentation of the corpuscular elements is *very* rapid.

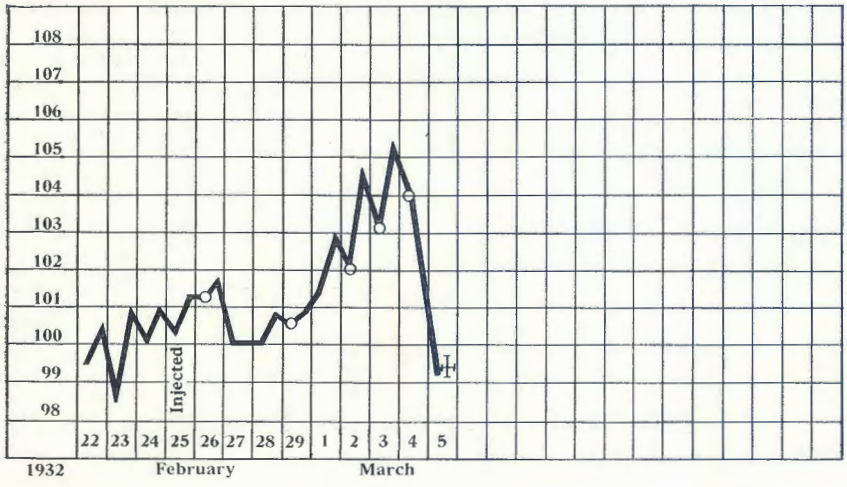
For the morphological aspect of equine blood, the red, white and differential counts, "red volume %" etc., I would refer to the works of Neser (1926) more particularly as the major part of those researches were also carried out here at Onderstepoort and the results obtained would, therefore, be more directly applicable to the bloods handled by me than if those researches had been carried out under different environmental conditions.

In presenting the experimental data of 14 horse-sickness cases the same sequence as previously will be adhered to, i.e., the temperature record (only for the actual hyperthermic period) on which small circles indicate the times of bleeding, followed by a history of the experimental subject, the analytical data and the main features emerging from the data collected. A statement of the normal values of all constituents determined will precede a general summary of the results obtained.

(*c*) Experimental Data.

Case I.

Horse 20302. *Horse-sickness* (*Dikkop*). *Died* 5/3/32.
Temperature Chart I.



History : Horse 20302. An aged, dark-chestnut stallion, in good condition ; was placed on temperature 11/12/31, and used for mosquito feeding experiments (Messrs. Bedford and du Toit), after being injected on 25/2/32 intrajugularly with 5 c.c. blood sent in by the Government Veterinary Officer, Eshowe. On 2/3/32 100 c.c. blood was drawn and mosquitos allowed to feed on this animal. On the fourth day p.i. the temperature reaction set in, reaching 105·6° within the following 72 hours, the temperature dropping by crisis, the animal succumbing on the 9th day p.i. from "Dikkop" horse-sickness. At post-mortem there was found to be present, transudation of the subcutis and loose connective tissue, cyanosis, slight hydrothorax, hydropericard, tumor splenis, subserous haemorrhages and slight verminosis of the digestive tract.

TABLE 1.

Horse 20302.

Date..... *Time*.....		26/2/32. —	29/2/32. —	2/3/32. —	3/3/32. —	4/3/32 —
Temporary Reactions.....		N	P.I.R.	R	R	R
Hb. gm. %.....		15·42	14·49	12·83	12·42	14·90
Sugar mgm. %.....	L	83·3	95·2	98·0	106·4	104·2
	U	67·6	77·0	77·0	88·5	96·2
T.N. gm. %.....		3·269	3·101	2·863	2·912	3·038
N.P.N. mgm. %.....	L	21·43	19·34	18·35	19·36	19·25
	U	14·89	14·63	14·00	14·81	15·11
Coag. N. gm. N %.....	L	3·248	3·083	2·845	2·893	3·019
	U	3·254	3·086	2·849	2·897	3·023
Urea mgm. N %	L	6·42	7·80	5·73	7·24	7·36
		13·44	16·38	11·97	15·12	15·54
	U	6·13	7·30	5·69	6·72	6·94
		12·81	16·38	11·97	14·07	14·49
Total Creatinine mgm. N %	L	2·36	2·11	1·86	1·82	1·82
		6·40	5·70	5·00	4·90	4·96
	U	1·86	1·78	1·60	1·45	1·93
		5·00	4·80	4·30	3·90	5·20
Uric acid mgm. N %	L	0·82	0·67	0·82	0·67	0·67
		2·46	2·00	2·45	2·00	2·00
	U	0·40	0·23	0·27	0·30	0·57
		1·20	0·70	0·80	0·90	1·71
Amino acid mgm. N %	L	7·80	6·44	6·14	7·00	7·00
	U	4·70	3·35	4·54	4·44	4·10
R.N. mgm. N %.....	L	4·03	2·67	3·80	2·63	2·40
	U	1·80	1·53	1·90	1·90	1·57
Plasma.....		—	—	—	—	—

Main Features of Data.

Hb.—Shows a drop from 15·42 to 12·42 gm. per cent. with a subsequent rise to 14·90 gm. per cent. on day prior to death.

Sugar.—This rises from 83 mgm. per cent. to 106·4 mgm. per cent. in the case of "laked" and from 67·6–96·2 mgm. per cent. in "unlaked" filtrates.

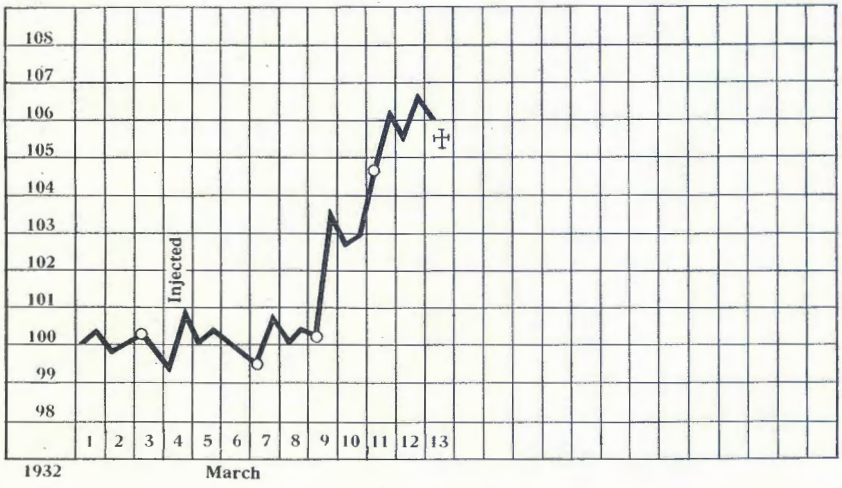
T.C.N.—A decrease from 2·36–1·82 mgm. N per cent. is noted in "laked" and from 1·86–1·45 mgm. N per cent. in "unlaked" filtrate, with a rise on day before death to 1·93 mgm. N per cent.

T.N.—A slight decrease running parallel with Hb. values.

U.P.N., *U.N.*, *U.A.N.*, *A.A.N.*, and *R.N.*—No changes in either direction are noted.

CASE II.

Horse 20262. *Horse-sickness* (*mixed type*). *Died* 13/3/32.
Temperature Chart II.



History: Horse 20262. A chestnut gelding, approximately 12 years old, in good condition. Was placed on temperature on 20/10/31, showing a regular normal temperature record. On the 4th of March this animal was injected intrajugularly with 5 c.c. blood from a mule (*ex* Agricultural School, Losperfontein). On the 5th day p.i. the temperature reaction set in reaching 106·4° within 48 hours of the onset. The animal died on 3/3/32, i.e., on the 9th day p.i. Anorexia, dyspnoea and swelling of the supra-orbital fossa were noted. The pathological anatomical findings were cyanosis of the mucous membranes, transudation of plasma into the subcutis, loose connective tissue throughout the body and subpleurally. Severe hydrothorax and oedema of the lungs, hydropericard, subendocardial haemorrhages, degeneration of the myocard, fatty degeneration and hyperaemia of the liver and kidneys and slight tumor splenis. The clinical and post-mortem findings suggest this to have been a case of the " mixed " type of horse-sickness.

TABLE 2.

Horse 20262.

Date		3/3/32.	7/3/32.	9/3/32.	11/3/32.
Time		—	—	—	—
Temperature Reactions		N	P.I.N.	R	R
Hb. gm. %		18·57	18·96	22·63	15·42
Sugar mgm. %	L	64·5	71·4	57·8	100·0
	U	58·5	62·1	47·8	83·3
T.N. gm. N %		3·437	3·465	3·465	3·031
N.P.N. mgm %	L	22·5	27·0	27·5	21·1
	U	16·5	17·8	17·5	15·3
Coag. N gm. N %	L	3·415	3·438	3·438	3·010
	U	3·420	3·447	3·448	3·016
Urea mgm. N %	L	7·30	9·00	9·50	9·90
		15·33	18·90	19·95	20·79
	U	6·60	9·00	9·00	9·40
		13·86	18·90	18·90	19·74
Total Creatinine mgm. N %	L	2·01	2·01	2·19	2·29
		5·40	5·40	5·84	6·20
	U	2·10	1·90	2·10	1·50
		5·70	5·10	5·68	4·00
Uric acid mgm. N %	L	0·53	0·53	0·58	0·50
		1·60	1·60	1·73	1·50
	U	0·53	0·30	0·47	0·20
		1·60	0·89	1·40	0·60
Amino acid mgm. N %	L	10·8	12·7	8·7	8·2
		5·8	3·9	4·5	3·7
R.N. mgm. N %	L	1·90	2·80	6·50	1·85
	U	1·50	2·72	1·40	0·50
Plasma		—	—	—	—

Salient Features of Analytical Data.

Hb.—Shows a rise from 18·57–22·63 gm. per cent., succeeded by a drop to 15·42 gm. per cent. 48 hours before death.

Sugar.— An increase is towards the end noted (±66–100 mgm. per cent.).

T.N.—A decrease is noted coinciding with the drop of Hb. value.

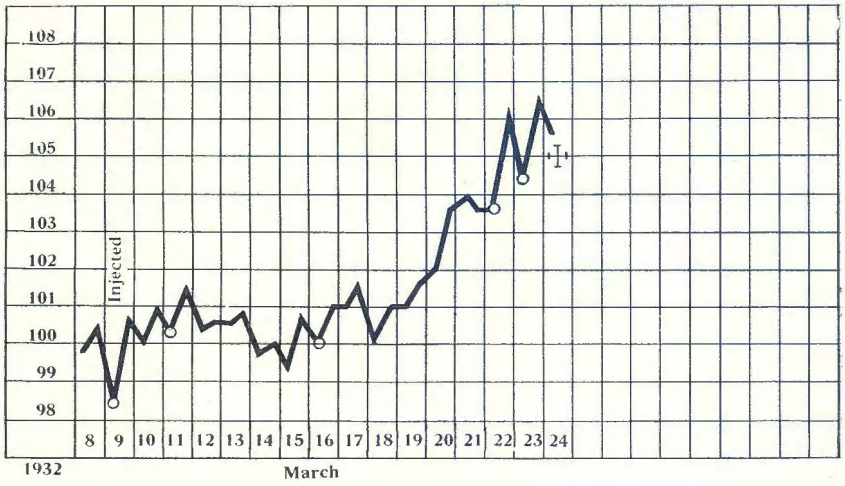
N.P.N.—A tendency towards a slight increase in the incubation period is succeeded by a drop 48 hours before death. The changes are, however, only within the normal variation.

U.N., *T.C.N.*, *U.A.N.*, *R.N.*—Nothing unusual.

A.A.N.—Shows a slight decrease (anorexia ?).

Case III.

Horse 20288. *Horse-sickness* (*mixed*). *Died* 24/3/32.
Temperature Chart III.



History: Horse 20288. Bay gelding, aged, in medium condition, was placed on temperature 11/12/31 (mosquito transmission experiments, Messrs. Bedford and du Toit). The temperature remained normal until 19/3/32, when a temperature reaction set in, the horse dying six days later from horse-sickness. This case is of interest in that horse-sickness was apparently produced through the subcutaneous injection of mosquitos on the 9th, 10th and 11th March, 1932. These mosquito transmission experiments will be dealt with by their authors (Messrs. Bedford and du Toit) in the next report of the Director of Veterinary Services, to which those interested are referred. The post-mortem findings substantiate the clinical diagnosis of horse-sickness. the pathological findings being transudation into subcutis, subpleural and loose connective tissue, slight hydropericard, extensive subendocardial haemorrhages, oedema of the lungs, hyperaemia and fatty degeneration of the liver and slight tumor splenis.

TABLE 3.

Horse 20288.

Date		9/3/32.	11/3/32.	16/3/32.	22/3/32.	23/3/32.
Time		—	—	—	—	—
Temperature Reactions		N	N	P.I.N.	R	R
Hb. gm. %		17·84	16·87	15·19	13·31	13·31
Sugar mgm. %	L	70·92	74·60	71·43	105·36	93·46
	U	57·50	54·30	58·48	91·76	87·72
T.N. gm. %		3·220	3·465	3·325	2·874	3·094
N.P.N. mgm. %	L	23·10	19·03	18·75	18·75	21·23
	U	17·80	15·00	15·00	15·15	16·86
Coag. N gm. N %	L	3·197	3·446	3·306	2·795	3·073
	U	3·202	3·450	3·310	2·799	3·077
Urea mgm. N %	L	10·70	8·53	6·57	7·00	8·09
		22·47	17·85	13·86	14·70	17·01
	U	10·30	8·91	6·63	7·16	8·09
		21·63	18·69	13·86	15·12	17·01
Total Creatinine mgm. N %	L	2·40	1·86	2·04	1·84	1·98
		6·40	5·00	5·50	4·96	5·32
	U	1·90	1·41	1·58	1·56	1·90
		5·14	3·70	4·24	4·22	5·14
Uric acid mgm. N %	L	0·71	0·64	0·63	0·44	0·44
		2·14	1·91	1·89	1·31	1·31
	U	0·70	0·20	0·30	0·39	0·36
		2·10	0·60	0·91	1·16	1·07
Amino acid mgm. N %	L	7·39	6·90	7·37	6·51	7·00
	U	4·10	3·50	4·00	3·50	3·50
R.N. mgm. N %	L	1·90	1·06	2·14	2·96	3·72
	U	0·80	1·98	2·49	2·54	3·00
Plasma		—	—	—	—	—

Main Features of Analytical Data.

Hb.—Drops from 17·84–13·31 gm. per cent.

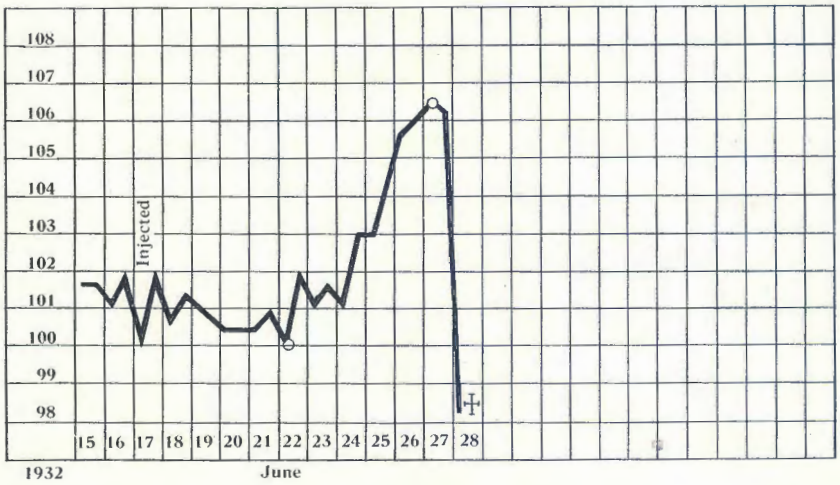
Sugar.—Increases from 70·92–105·36 mgm. per cent. and drops 24 hours before death to 93·46 mgm. per cent.

T.N.—Tending to drop slightly.

N.P.N., *U.N.*, *T.C.N.*, *U.A.N.*, *A.A.N.* and *R.N.*—Show no marked or definite alterations but in all there is a *slight* tendency towards a decrease.

Case IV.

Horse 20276. *Horse-sickness* (*Dunkop*). *Died* 28/6/32.
Temperature Chart IV.



History: Horse 20276. A light bay gelding, 12 years old, in poor condition. Was placed on temperature 23/12/31 and utilised in mosquito transmission experiments (Messrs. Bedford and du Toit). The temperature remained normal throughout. The horse was injected on 17/6/32 intrajugularly with 2 c.c. blood from mule 6396 (*ex* Agricultural Training School, Losperfontein). The temperature reaction set in on the 7th day p.i. the horse succumbing three days later. On the 27th June the horse received 1 gm. Acriflavin (Mr. Parkin) shortly *after* the blood was drawn for analyses. At the post-mortem examination there was found to be present the characteristic pathological changes associated with horse-sickness, e.g., transudation into subcutis and loose connective tissue, hydropericard, marked oedema of the lungs, a slight fatty degeneration change of the liver and pigmentation of the renal cortex.

Horse 20276. TABLE 4.

Date / *Time*		15/4/32. 11 a.m.	26/4/32. —	3/6/32. 7.15 a.m.	6/6/32. —	9/6/32. —	14/6/32. —	22/6/32. —	27/6/32. —
Temperature Reaction		N	N	N	N	N	N	P.I.N.	R
Hb. gm. %		14·72	14·08	17·51	13·31	14·72	15·19	15·42	19·33
Sugar mgm. %	L	89·28	97·74	66·67	85·47	83·33	—	75·75	60·98
	U	80·65	83·83	57·14	76·34	73·56	—	66·67	55·55
T.N. gm. %		3·172	2·976	3·242	2·780	2·892	2·990	3·004	3·291
N.P.N. mgm. %	L	23·08	24·04	19·36	19·37	18·65	18·75	20·98	20·00
	U	18·50	18·61	14·56	13·82	14·28	14·32	15·87	17·65
Coag. N gm. N %	L	3·149	2·952	3·223	2·761	2·873	2·971	2·983	3·271
	U	3·154	2·957	3·227	2·767	2·878	2·976	2·988	3·273
Urea mgm. N %	L	9·25	9·87	6·49	6·02	7·00	6·24	7·70	7·44
		19·53	20·79	13·65	12·60	14·70	13·02	16·17	15·54
	U	8·52	8·90	6·35	5·66	6·50	5·45	7·40	7·83
		17·85	18·69	13·44	11·97	13·65	11·55	15·54	16·38
Total Creatinine mgm. N %	L	2·42	2·42	2·42	2·01	2·23	—	2·50	2·76
		6·54	6·54	6·54	5·40	6·30	—	6·74	7·41
	U	2·23	2·23	2·23	2·23	2·10	—	2·23	2·66
		6·00	6·00	6·00	6·00	5·68	—	6·00	7·20
Uric acid mgm. N %	L	0·60	0·24	0·23	0·30	0·30	—	0·27	0·29
		1·80	0·73	0·69	0·69	0·91	—	0·80	0·87
	U	0·43	0·21	0·18	0·25	0·23	—	0·16	0·32
		1·28	0·62	0·55	0·75	0·69	—	0·48	0·97
Amino acid mgm. N %	L	7·78	8·24	5·83	6·67	6·33	7·45	6·19	7·14
	U	4·83	4·52	4·00	3·59	3·67	4·95	4·62	5·00
R.N. mgm. N %	L	3·03	3·33	4·39	4·44	2·79	5·06*	4·32	2·37
	U	2·50	2·75	1·86	2·09	1·78	3·97*	1·46	1·84
Plasma		n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	Slight orange

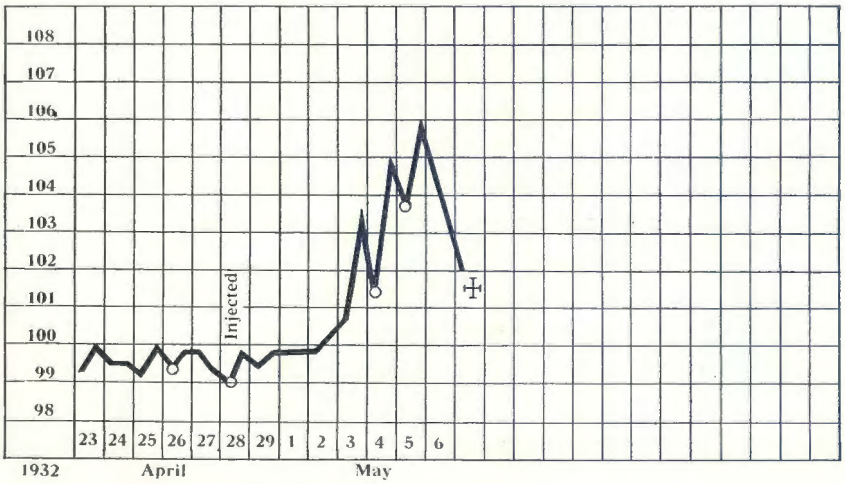
* Includes "Total Creatinine N" and "Uric Acid N."

Main Features of Analytical Data.

Hb.—An increased Hb. content at the height of the reaction a day before death is noted.
Sugar.—The lowest figure is recorded at height of reaction (for both filtrates).
T.N., *N.P.N.*, *U.N.*, *T.A.N.*, and *R.N.*—Nothing specific—variations within normal range.
T.C.N.—Shows an increase from about 2·30–2·76 mgm. N per cent. in "laked" and from 2·15–2·66 mgm. N per cent. in "unlaked" filtrate.

CASE V.

Horse 20130. *Horse-sickness* (*Dikkop*). *Died* 6/5/32.
Temperature Chart V.



History: Horse 20130. Bay gelding, 8 years old and in medium condition. Was placed on temperature 7/8/31 and used in mosquito transmission experiments (Messrs. Bedford and du Toit). Except for a slight, indefinite temperature reaction during the 6th to the 10th April, 1932, the temperature record was normal. On the 28th April, 1932, 1 c.c. blood from Horse 20302, Eschowe strain (*vide*) was injected subcutaneously. The temperature reaction set in on the fourth day p.i. The symptoms shown were those of a "Dikkop" horse-sickness reaction.

At the post-mortem examination (P.M. No. 11033 of 6/5/32) there was found a generalised cyanosis, transudation into the subcutis and loose connective tissue, hydrothorax, hydropericard, subepicardial and subendocardial haemorrhages, degeneration of the myocard, hyperaemia and slight fatty degeneration of the liver.

TABLE 5.

Date		14/3/32.	17/3/32.	23/3/32.	12/4/32.	26/4/32.	28/4/32.	3/5/32.	4/5/32.	6/5/32.*
Time		—	—	—	—	—	—	—	—	—
Temperature Reactions		N	N	N	N	N	N	R	R	R
Hb. gm. %		15·42	17·84	15·42	18·20	20·18	15·42	14·84	13·87	25·24
Sugar mgm. %	L	82·60	101·00	93·46	57·47	119·04	94·34	117·64	161·30	129·88
	U	66·20	80·65	89·30	—	84·03	71·94	105·26	142·86	103·10
T.N. gm. %		3·374	3·388	2·968	3·654	3·535	3·290	2·968	2·912	4·060
N.P.N. mgm. %	L	24·40	25·27	26·64	26·08	27·77	26·08	25·00	18·75	56·54
	U	18·70	18·20	17·54	18·75	20·08	17·85	18·61	16·66	47·62
Coag. N gm. N %	L	3·350	3·363	2·942	3·628	3·508	3·264	2·943	2·893	4·003
	U	3·355	3·370	2·950	3·635	3·515	3·272	2·949	2·895	4·012
Urea.....mgm. N %	L	12·30	9·20	10·11	10·11	12·33	10·43	10·48	8·90	21·08
„ U %		25·83	19·32	21·21	21·21	25·83	21·84	22·05	18·69	44·31
mgm. N %	U	11·50	8·52	9·53	9·00	11·34	9·58	10·11	8·80	20·40
„ U %		24·15	17·85	19·95	18·90	23·73	20·16	21·21	18·48	42·84
*Total Creatinine*mgm. N %	L	2·40	2·01	2·23	2·33	2·23	1·89	2·23	1·97	4·91
„ TC %		6·60	5·40	6·00	6·26	6·00	5·14	6·00	5·32	13·48
mgm. N %	U	2·00	1·75	1·89	2·04	2·23	1·49	1·78	1·41	—
„ TC %		5·40	4·70	5·14	5·54	6·00	4·00	4·80	3·80	—
Uric acid.....mgm. N %	L	0·30	0·39	0·27	0·43	0·29	0·41	0·28	0·25	0·63
„ UA %		0·84	1·16	0·81	1·32	0·87	1·22	0·84	0·74	1·88
mgm. N %	U	0·13	0·19	0·20	—	0·18	0·27	0·19	0·18	—
„ UA %		0·38	0·58	0·61	—	0·53	0·80	0·58	0·53	—
Amino acid mgm. N %	L	7·40	7·78	7·37	6·86	8·75	7·61	8·75	6·36	14·00
	U	3·70	4·52	3·68	3·89	4·12	4·12	3·89	4·12	11·66
R.N. mgm. N %	L	2·00	5·89	6·66	5·35	4·19	5·74	4·26	1·47	14·92
	U	1·51	3·22	2·24	3·00†	2·21	2·39	3·74	2·15	15·56‡
Plasma		—	—	—	—	—	—	—	—	—

* Animal down—blood drawn about four hours before death. † Includes " Uric Acid N." ‡ Includes " Uric Acid N " and " Creatinine N."

Main Features of Analytical Data.

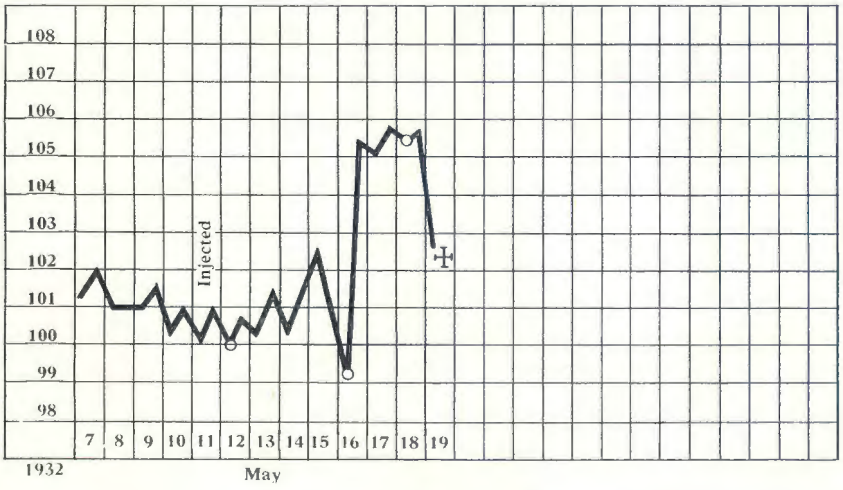
Hb.—High Hb. content in blood drawn on last day four hours before death. The animal was down and already in agonal stage.

Sugar.—Shows a steady rise in the " laked " filtrate to 161·30 mgm. per cent. the day before death, with decrease to 129·88 mgm. per cent. four hours before death; similar changes in " unlaked " filtrate.

T.N., *N.P.N.*, *U.N.*, *T.C.N.*, *U.A.N.*, *A.A.N.*, and *R.N.*—All these constituents show no changes—outside the limits of normal variation—except in the blood drawn on 6/5/32 four hours before death, in which they are all greatly increased in amount, in most cases more than doubled.

Case VI.

Horse 20300. *Horse-sickness* (*Dunkop*). *Died* 19/5/32.
Temperature Chart VI.



History: Horse 20300. A dark chestnut mare, 7 years old, in fair condition. Was placed on temperature on 21/3/32, being used for mosquito transmission experiments (Messrs. Bedford and du Toit). On the 11th of May, 1932, this animal received 5 c.c. blood intrajugularly from horse 20288 (vide). On the fifth day p.i. the temperature reaction set in, rising to 105·2° within 12 hours, the animal succumbing three days later from Dunkop horse-sickness. The post-mortem findings were cyanosis of the mucous membranes, oedema and hyperaemia of the lungs, hydropericard, subepicardial and sub-endocardial haemorrhages, general passive hyperaemia of all the organs, ascaris and habronema infection.

Horse 20300. TABLE 6.

Date / *Time*		18/4/32. —	19/4/32. —	22/4/32. —	25/4/32. —	27/4/32. —	6/5/32. —	12/5/32. —	16/5/32. —	18/5/32. —
Temperature Reaction		N	N	N	N	N	N	P.I.N.	R	R
Hb. gm. %		14·95	15·42	16·66	14·95	15·98	17·18	19·33	19·42	16·87
Sugar mgm. %	L	96·15	—	84·74	102·04	97·09	84·03	102·04	94·34	99·91
	U	71·43	—	66·67	86·96	71·43	80·00	69·93	73·56	67·11
Total Nitrogen gm. %		2·808	3·116	3·032	2·920	3·200	3·354	3·354	3·179	2·934
N.P.N. mgm. %	L	19·36	22·94	20·54	20·83	20·68	21·14	21·43	18·75	20·00
	U	15·00	15·28	15·30	13·63	14·92	15·00	15·28	13·63	14·62
Coag. N gm. %	L	2·789	3·094	3·011	2·899	3·179	3·333	3·333	3·160	2·914
	U	2·793	3·101	3·017	2·906	3·185	3·339	3·339	3·165	2·919
Urea mgm. N %	L	5·89	7·33	6·27	4·70	7·00	6·46	6·84	6·02	6·63
„ U %		12·39	15·33	13·23	9·87	14·70	13·65	14·28	12·60	13·86
mgm. N %	U	5·78	6·69	6·13	4·40	6·69	6·12	6·40	5·66	6·40
„ U %		12·18	14·07	12·81	9·24	14·07	12·81	13·44	11·97	13·44
Total Creatinine mgm. U %	L	2·06	1·91	1·67	1·52	1·91	1·98	2·04	2·01	1·82
„ TC %		5·54	5·14	4·50	4·10	5·14	5·32	5·50	5·40	4·90
mgm. N %	U	1·40	1·26	1·33	1·01	1·49	1·61	1·49	1·51	1·45
„ TC %		3·78	3·40	3·60	2·66	4·00	4·32	4·00	4·08	3·92
Uric acid mgm. N %	L	0·41	0·44	0·41	0·41	0·58	0·48	0·51	0·43	0·50
„ UA %		1·22	1·31	1·22	1·22	1·73	1·45	1·52	1·29	1·50
mgm. N %	U	0·31	0·37	0·31	0·18	0·42	0·37	0·20	0·32	0·22
„ UA %		0·94	1·10	0·94	0·55	1·25	1·12	0·61	0·97	0·66
Amino acid mgm. N %	L	8·24	9·33	9·79	8·00	8·75	8·24	8·75	7·49	8·24
	U	4·67	4·52	4·38	4·38	3·89	5·83	5·00	4·00	3·89
R.N. mgm. N %	L	2·78	3·93	2·40	6·20	2·44	3·99	3·29	2·80	2·81
	U	2·84	2·44	3·15	3·66	2·53	1·07	2·18	2·14	1·66
Plasma		n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	Pale orange

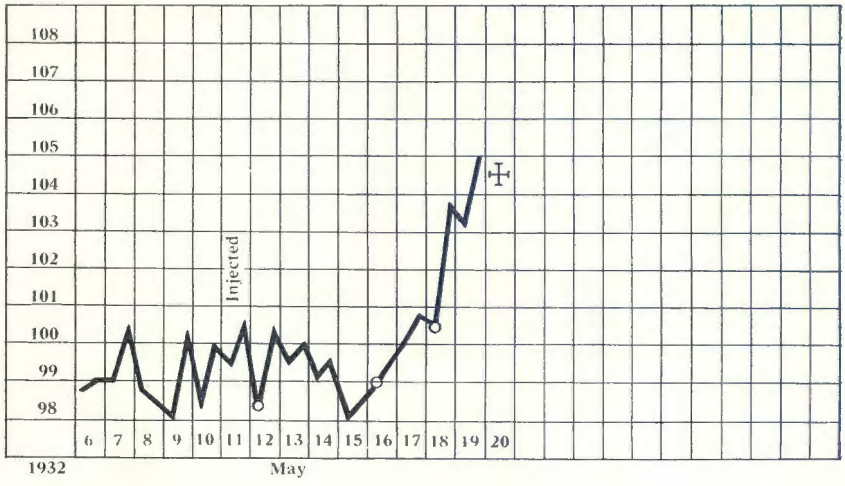
Main Features of Analytical Data.

Hb.—Variable, but highest Hb. content is during part of incubation period and at initial stage of the temperature reaction, dropping slightly on day before death.

Sugar, T.N., N.P.N., U.N., T.C.N., U.A.N., A.A.N., and *R.N.*—Nothing abnormal noted.

Case VII.

Horse 20297. *Horse-sickness* (*Dikkop*). *Died* 20/5/32.
Temperature Chart VII.



History: Horse 20297. 14 year old bay gelding, fairly poor condition, was placed on temperature on 11/12/31 and used for various mosquito transmission experiments (Messrs. du Toit and Bedford). The temperature remained normal throughout. On 11/5/32 the horse was injected with 5 c.c. blood from mule 6396. The temperature reaction set in on the fifth day, reaching 105° on the eighth day p.i., the animal succumbing at about 10 a.m. on 20/5/32. The patient showed typical dikkop horse-sickness symptoms. The pathological anatomical findings included a slight general anaemia, numerous subendocardial haemorrhages, hyperaemia of the lungs, marked degeneration of the liver, slight strongylosis, and a heavy infection with trichonema, slight gastritis and enteritis catarrhalis.

TABLE 7.

Horse 20297.

Date.....		15/4/32.	27/4/32.	12/5/32.	16/5/32.	18/5/32.	20/5/32.
Time.....		11 a.m.	—	—	—	—	*
Temp. R.....		N	N	P.I.N.	P.I.N.	R	R
Hb. gm. %.....		11·67	13·68	13·68	18·11	14·28	23·18
Sugar mgm. %	L	75·19	111·10	105·23	94·34	80·65	77·52
	U	—	95·24	91·75	79·36	73·56	55·87
T.N. gm. %.....		2·794	2·738	2·710	3·025	2·696	—
N.P.N.	L	27·27	25·00	23·71	23·08	21·43	33·72
mgm. %	U	—	19·75	16·48	18·41	15·00	25·74
Coag. N.	L	2·767	2·713	2·686	3·002	2·675	—
gm. N %	U	—	2·718	2·694	3·007	2·681	—
Urea mgm. N %	L	13·29	11·65	8·90	10·83	8·17	12·42
		27·93	24·57	18·69	22·68	17·22	26·04
	U	—	12·51	8·56	10·11	7·47	12·16
		—	26·25	18·06	21·21	15·75	25·62
	L	2·80	2·42	2·23	2·42	2·27	2·42
Total Creatinine		7·58	6·54	6·00	6·54	6·16	6·54
mgm. N %	U	—	1·82	1·75	2·01	1·61	2·04
		—	4·90	4·70	5·40	4·32	5·50
Uric acid mgm.	L	0·70	0·44	0·37	0·36	0·30	0·58
N %		2·09	1·31	1·10	1·07	0·89	1·75
	U	—	0·38	0·21	0·30	0·27	0·34
		—	1·14	0·64	0·91	0·82	1·02
Amino acid	L	7·37	8·24	7·00	7·00	6·36	8·00
mgm. N %	U	—	4·38	5·04	3·89	3·68	5·04
R.N.	L	3·11	1·39	5·21	2·47	4·33	10·30
mgm. N %	U	—	1·54	0·92	2·10	1·97	6·16
Plasma.....		n.u.	n.u.	n.u.	slightly orange	orange	orange

* Drawn one hour before death.

Main Features of Analytical Data.

Hb.—Shows normal variation, except on day of death when the Hb. value has become increased to 23·18 gm. per cent.

Sugar.—No change.

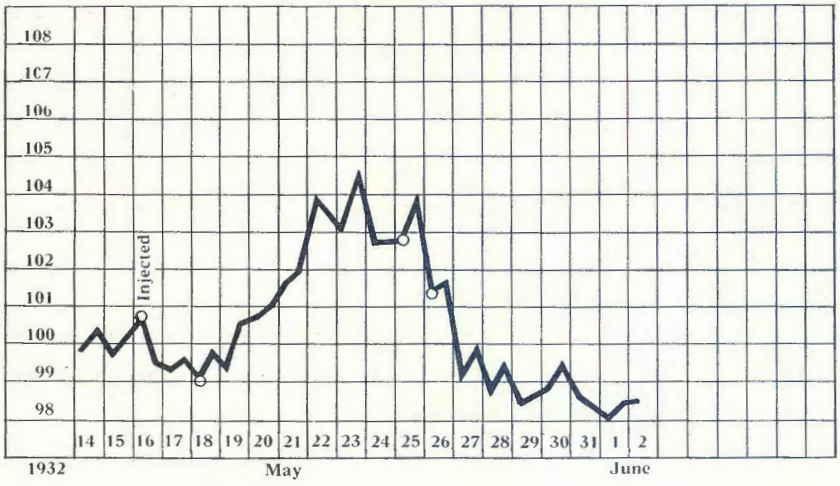
N.P.N.—Except for a slight increase in the blood drawn shortly before death, no definite changes are noticeable.

R.N.—This N fraction is markedly increased just before death.

U.N., *T.C.N.*, *U.A.N.*, *A.A.N.* and *T.N.*—Show no alterations.

CASE VIII.

Horse 20308. *Horse-sickness* (*Recovered*), *May/June*, 1932.
Temperature Chart VIII.



History: Horse 20308. Dark brown gelding, about 8 years old, in fair condition. Was placed on temperature 14/3/32 and used for various immunisation experiments (Mr. Alexander). On 16/5/32 it received intrajugularly 5 c.c. blood from Horse 20065 (O. virus 191st generation). The temperature reaction set in after four days, lasting for eight days, when the temperature had returned to normal. The horse did not show marked clinical symptoms, only a light anorexia lasting for two to three days. After recovering, the animal was kept on temperature and is still under observation at the moment.

Horse 20308. TABLE 8.

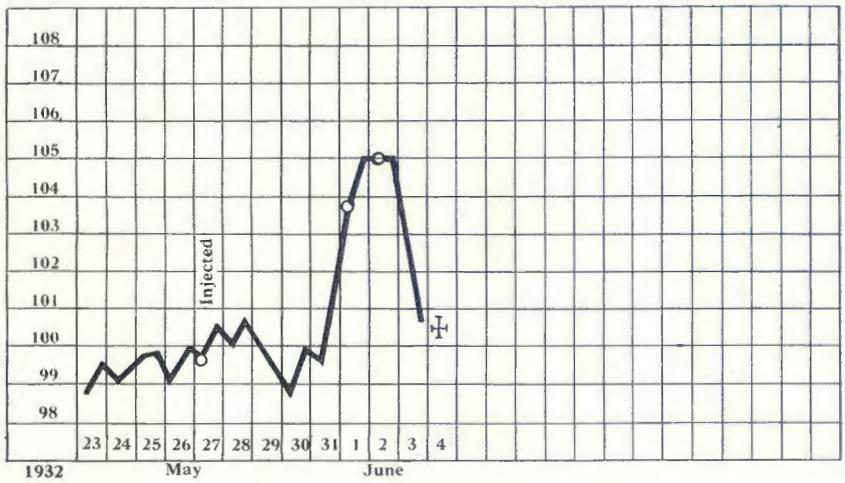
Date / *Time*		12/5/32. —	13/5/32. —	16/5/32. —	18/5/32. —	25/5/32. —	26/5/32. —	3/6/32. —
Temperature Reaction		N	N	N	P.I.N.	R	R	N
Hb. gm. %		16·87	16·87	14·72	15·19	12·94	11·33	15·19
Sugar mgm. %	L	81·97	73·60	74·63	75·19	75·19	73·00	71·94
	U	66·23	59·52	59·52	64·94	61·35	65·79	62·11
T.N. gm. %		3·130	3·186	3·102	3·200	2·710	2·598	3·018
N.P.N. mgm. %	L	22·06	27·27	24·62	22·72	18·93	17·65	21·06
	U	16·30	21·43	18·65	16·66	14·42	14·28	14·56
Coag. N gm. N %	L	3·108	3·159	3·077	3·177	2·691	2·580	2·997
	U	3·114	3·165	3·083	3·183	2·696	2·584	3·003
Urea mgm. N %	L	9·25	11·58	10·83	8·85	6·84	7·33	6·17
		19·53	24·15	22·68	18·69	14·28	15·33	12·81
	U	9·00	10·90	10·77	8·43	6·75	6·84	5·76
		18·90	22·89	22·68	17·64	14·07	14·28	12·18
Total Creatinine mgm. N %	L	2·10	1·87	2·50	2·27	2·10	2·23	1·97
		5·68	5·02	6·74	6·16	5·68	6·00	5·32
	U	1·58	1·61	1·82	1·90	1·61	1·87	1·54
		4·24	4·32	4·90	5·14	4·32	5·02	4·16
Uric acid mgm. N %	L	0·25	0·25	0·24	0·28	0·25	0·21	0·21
		0·76	0·76	0·74	0·85	0·76	0·62	0·64
	U	0·18	0·24	0·17	0·17	0·15	0·15	0·12
		0·53	0·71	0·51	0·51	0·46	0·44	0·36
Amino acid mgm. N %	L	7·00	8·33	7·78	8·43	7·00	5·83	6·09
	U	4·00	4·24	4·12	4·00	3·68	3·50	3·78
R.N. mgm. N %	L	3·56	5·24	3·26	2·69	2·74	2·05	6·52
	U	1·54	4·44	1·77	2·19	2·23	1·93	4·36
Plasma		n.u.	n.u.	n.u.	Orange	Slight orange	Pale orange	n.u.

Main Features of Analytical Data.

Hb.—The lowest Hb. values are found during the actual reaction rising to normal shortly after the return of temperature to normal.
U.N.—Shows a slight tendency to decrease.
A.A.N.—Shows a slight tendency to decrease.
R.N.—This is highest shortly after return of temperature to normal, but the amount of N. is within the normal range and this finding is probably merely coincidental.
N.P.N., *Sugar*, *T.C.N.*, *U.A.N.*, and *T.N.*—Show no change in either direction.

CASE IX.

Horse 20315. *Horse-sickness* (*mixed type*). *Died* 4/6/32.
Temperature Chart IX.



History: Horse 20315. An aged bay gelding, in good condition. Was placed on temperature 29/3/32 and was used in various mosquito transmission experiments (Messrs. Bedford and du Toit) and on 27/5/32 received intra-jugularly 5 c.c. blood from Horse 20302 (*vide*). On the 3rd day p.i., the temperature reaction set in, rising to 105° within 24 hours, the horse succumbing on the 7th day p.i. from mixed type of horse-sickness. At the post-mortem examination was found cyanosis of the mucous membrane, transudation into the subcutis and loose connective tissue, marked oedema and hyperaemia of the lungs, subepicardial and subendocardial haemorrhages and degeneration of the myocard, stasis of the liver, strongylosis infection of the colon, and fatty degeneration of the kidneys.

TABLE 9.

Horse 20315.

Date		20/4/32.	4/5/32.	27/5/32.	1/6/32.	2/6/32.
Time		—	—	—	—	7.15 a.m.
Temperature Reactions		N	N	N	R	R
Hb. gm. %		16·29	11·67	10·60	10·27	10·81
Sugar mgm. %	L	80·65	80·50	91·74	109·90	96·15
	U	72·46	77·52	82·64	96·15	84·74
T.N. gm. %		3·116	2·710	2·402	2·430	2·437
N.P.N. mgm. %	L	25·00	23·08	23·08	21·43	18·60
	U	18·75	18·07	19·11	16·21	14·42
Coag. N gm. N %	L	3·091	2·687	2·379	2·409	2·418
	U	3·097	2·692	2·383	2·414	2·423
Urea mgm. N %	L	12·33	10·77	11·12	9·00	7·89
		25·83	22·68	23·31	18·90	16·59
	U	11·50	10·24	10·72	8·75	7·73
		24·15	21·42	22·47	18·27	16·17
Total Creatinine mgm. N %	L	2·06	1·91	2·27	1·78	1·91
		5·54	5·14	6·16	4·80	5·14
	U	1·91	1·67	2·01	1·67	1·61
		5·14	4·50	5·40	4·50	4·32
Uric acid mgm. N %	L	0·30	0·23	0·24	0·25	0·19
		1·91	0·69	0 73	0·76	0·57
	U	—	0·15	0·15	0·23	0·11
		—	0·46	0·45	0·69	0·34
Amino acid mgm. N %	L	8·54	7·00	6·51	6·67	5·00
	U	5·38	4·38	4·38	3·89	2·80
R.N. mgm. N %	L	1·77	3·17	2·94	3·73	3·61
	U	—	1·63	1·85	1·67	2·18
Plasma		n.u.	n.u.	n.u.	n.u.	Slightly orange

Main Features of Analytical Data.

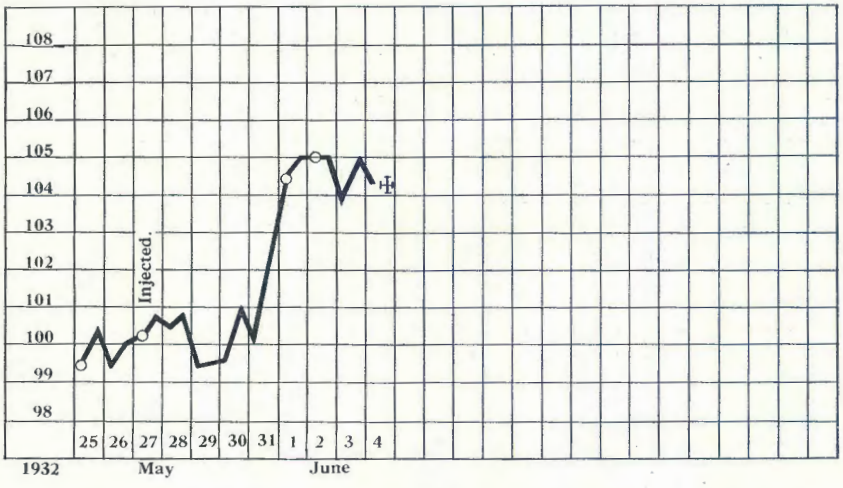
Sugar.—Shows a slight increase at beginning of temperature reaction.

N.P.N., *U.N.*, and *A.A.N.*—Show a slight decrease towards approach of death.

H.B., *T.C.N.*, *U.A.N.*, *R.N.*, and *T.N.*—No changes noted.

CASE X.

Horse 20280. *Horse-sickness* (*Dikkop*). *Died* 4/6/32.
Temperature Chart X.



History : Horse 20280. Chestnut gelding, six years old, in good condition and of a somewhat wild temperament. Placed on temperature on 24/11/31 and used in mosquito transmission experiments. The temperature remained normal throughout. On 27/5/31 the animal was injected subcutaneously with 1 c.c. blood from Horse 20302 (*vide*). On the 4th day p.i., the temperature reaction set in, the horse succumbing on the 9th day p.i. from Dikkop horse-sickness. At the post-mortem examination marked putrefactive changes were already found to be present, but the chief changes associated with horse-ickness could still be determined, such as gelatinous transudation, subserous petichiae, subendocardial haemorrhages and hydropericard.

Horse 20280.

TABLE 10.

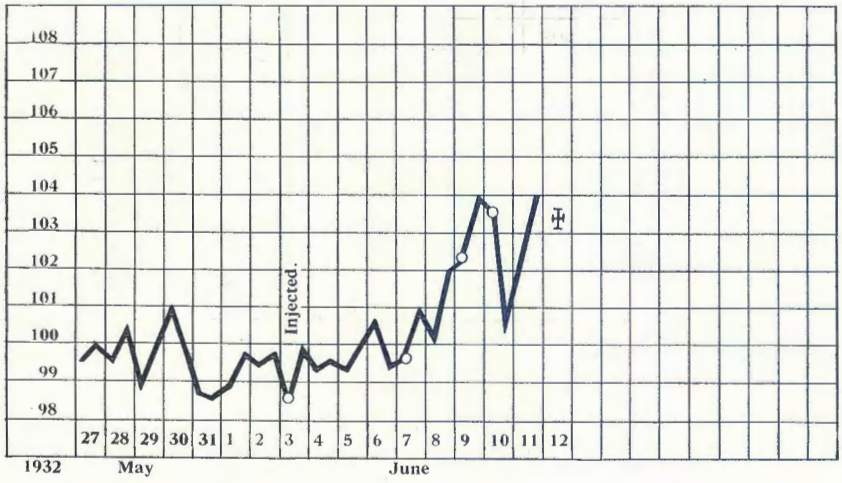
Date	30/11/31.	4/12/31.	4/3/32.	9/3/32.	11/3/32.	16/3/32.	20/4/32.	25/5/52.	27/5/32.	1/6/32.	2/6/32.
Time	—	—	—	—	—	—	—	—	—	—	7.15 a.m.
Temperature Reactions	N	N	N	N	N	N	N	N	N	R	R
Hb. gm. %	13·31	13·25	15·42	16·87	15·19	15·19	15·21	18·20	16·87	14·95	—
Sugar mgm. % L	92·60	84·74	90·91	82·64	106·40	100·00	97·07	80·00	90·09	—	117·64
U	81·97	69·94	91·75	70·92	83·30	86·96	83·33	50·00	81·30	—	102·04
T.N. gm. %	2·964	2·850	2·989	3·248	3·115	3·325	3·144	3·479	3·507	3·186	—
N.P.N. mgm. % L	26·08	22·25	23·55	25·60	23·10	23·10	25·00	22·81	21·90	20·71	20·57
U	22·40	16·94	18·60	20·00	17·00	16·72	18·75	16·51	17·65	13·95	13·32
Coag. N mg. N % L	2·938	2·828	2·965	3·222	3·092	3·302	3·119	3·456	3·485	3·165	—
U	2·942	2·833	2·970	3·228	3·098	3·308	3·125	3·462	3·409	3·172	—
Urea ..... mgm. N % L	12·30	9·60	10·10	11·50	9·50	7·44	11·50	8·30	9·10	6·75	6·52
„ U %	25·83	20·16	21·21	24·15	19·95	15·54	24·15	17·43	19·11	14·28	13·65
mgm. N % .... U	12·20	9·20	10·00	11·30	9·30	7·44	10·72	8·30	8·52	6·52	6·35
„ U %	25·62	19·32	21·00	23·73	19·63	15·54	22·47	17·43	17·85	13·65	13·44
Total Creatinine											
mgm. N % L	2·48	2·48	2·11	2·30	2·23	2·42	2·13	2·23	2·66	2·11	2·27
„ TC % ...	6·70	6·70	5·68	6·20	6·00	6·54	5·76	6·00	7·20	5·67	6·16
mgm. N % .... U	2·00	2·20	2·10	2·10	1·67	1·91	1·78	1·54	2·17	1·62	1·61
„ TC % ...	5·54	6·00	5·68	5·70	4·50	5·14	4·80	4·16	5·84	4·32	4·32
Uric acid ... mgm. N % L	0·44	0·48	0·37	0·37	0·34	0·37	0·41	0·34	0·30	0·38	0·25
„ UA % ..	1·33	1·43	1·10	1·10	1·03	1·10	1·24	1·03	0·91	1·05	0·75
mgm. N % .... U	0·21	0·21	0·30	0·25	0·16	0·23	0·22	0·16	0·15	0·23	0·13
„ UA % ..	0·64	0·64	0·89	0·75	0·48	0·69	0·67	0·49	0·46	0·69	0·40
Amino acid mgm. % L	6·67	7·00	7·80	9·03	8·43	8·24	10·00	8·75	7·78	6·25	5·60
U	5·71	4·38	4·20	4·40	4·40	4·52	4·83	3·89	4·67	4·00	2·50
R.N. mgm. N % L	4·20	2·69	3·18	2·40	2·60	4·63	1·00	3·19	2·06	4·22	5·93
U	2·30	1·00	2·00	1·95	1·47	2·62	0·20	2·62	2·14	1·59	2·74
Plasma	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	n.u.	Orange	Orange	Slightly orange

Main Features of Analytical Data.

Sugar.—Shows an increase during the temperature reaction.
U.N., *U.A.N.*, and *A.A.N.*—A relatively slight drop is noted just prior to death.
R.N.—A slight increase is noted.
Hb., *N.P.N.*, *T.C.N.*, and *T.N.*—No changes noted.

CASE XI.

Horse 20291. *Horse-sickness* (*Dunkop*). *Died* 12/6/32.
Temperature Chart XI.



History: Horse 20291. A black gelding, nondescript breed. Aged and in poor condition. The horse was placed on temperature 11/12/31. This horse was then used for mosquito transmission experiments (Messrs. Bedford and du Toit) and with the exception of a slight temperature reaction from the 15th to the 24th of February, 1932, of an undetermined nature, showed a very even normal temperature course. On 3/6/32, 1 c.c. blood was injected from Horse 20302 (Kaalplaas strain). The symptoms shown were those of a typical Dunkop horse-sickness reaction, the temperature reaction beginning on the 4th day p.i. On 10/6/32, 0·002 gm. Aciron per Kg. were injected for experimental purposes (Mr. Parkin) which was followed within 12 hours by a drop of temperature from 104°–100·4°, but during the next 24 hours the temperature rose again to 104°, the animal dying on the 12th of June (i.e., the 9th day p.i). On post-mortem examination was found to be present cyanosis of the mucous membranes, transudation into the subcutis, marked hydrothorax, hydropericard, severe oedema and hyperaemia of the lungs, subendocardial haemorrhages, degeneration of the myocard, fatty degeneration and hyperaemia of the liver and slight post-mortem changes of the kidneys.

TABLE 11.

Horse 20291.

Date.....	20/4/32.	26/4/32.	3/6/32.	7/6/32.	9/6/32.	10/6/32.
Time.....	—	—	7.15 a.m.	—	—	—
Temp. R.....	N	N	N	P.I.N.	R	R
Hb. gm. %.....	14·72	17·18	14·08	11·39	11·39	12·59
Sugar mgm. % L	82·64	108·70	70·42	77·52	102·10	—
U	70·42	100·00	60·61	66·67	90·91	—
T.N. gm. %.....	2·654	3·116	2·906	2·584	2·584	2·682
N.P.N. L	28·85	28·56	21·43	24·59	22·75	20·98
mgm. % U	21·75	20·83	15·79	19·14	18·07	15·28
Coag. N L	2·625	3·087	2·885	2·559	2·561	2·661
gm. % U	2·632	3·095	2·890	2·565	2·566	2·667
Urea						
mgm. N % L	13·18	11·50	6·87	11·20	10·24	7·77
,, U %	27·72	24·15	14·49	23·52	21·42	16·38
mgm. N % U	12·33	10·77	7·06	10·72	9·52	7·62
,, U %	25·83	22·68	14·91	22·47	19·95	15·96
T.C.						
mgm. N % L	1·98	1·78	2·27	2·42	1·98	—
,, TC %	5·32	4·80	6·16	6·54	5·32	—
,, N % U	1·78	1·49	1·78	2·01	1·67	—
,, TC	4·80	4·00	4·80	5·40	4·50	—
Uric acid						
mgm. N % L	0·62	0·45	0·38	0·37	0·38	—
,, UA %	1·86	1·34	1·14	1·12	1·14	—
mgm. N % U	0·37	0·27	0·22	0·29	0·27	—
,, UA	1·10	0·80	0·65	0·87	0·80	—
Amino acid L	9·33	8·75	5·74	5·83	6·01	5·71
mgm N % U	5·49	5·18	3·50	3·50	3·16	3·18
R.N. mgm. N % L	3·74	6·08	6·17	4·77	4·14	7·50*
U	1·78	3·12	3·23	2·62	3·45	4·48*
Plasma.....	—	—	—	—	—	—

* Includes "Total Creatinine Nitrogen" and "Uric Acid Nitrogen."

Main Features of Analytical Data.

Hb.—A slight decrease is observed.

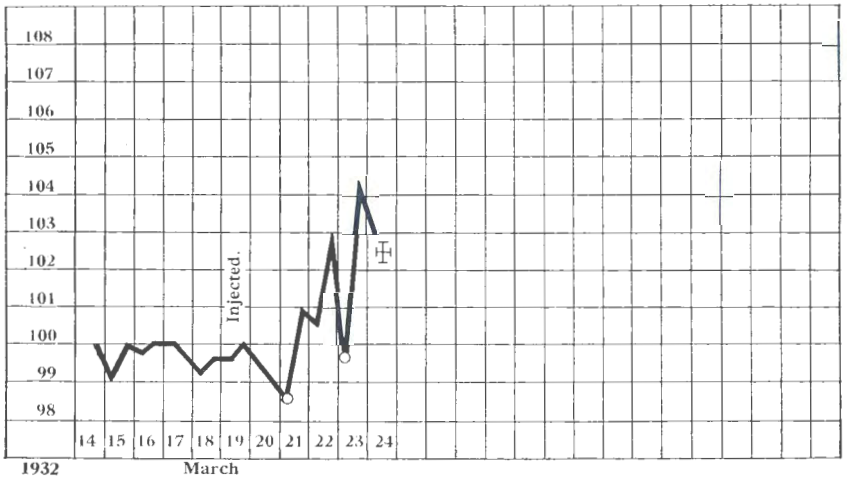
Sugar.—A slight increase is observed.

R.N.—In this particular case the R.N. throughout the period of examination is on the high side for both filtrates. The horse was several times clinically examined, but no explanation can be offered for the relatively high R.N. found.

N.P.N., *U.N.*, *T.C.N.*, *U.A.N.*, *A.A.N.*, and *T.N.*—No changes noted.

CASE XII.

Horse 20270. *Horse-sickness* (*mixed type*). *Died* 24/3/32.
Temperature Chart XII.



History: Horse 20270. An aged bay gelding, in fair condition. Placed on temperature 23/11/31 and used for mosquito transmission experiments (Messrs. Bedford and du Toit). The temperature remained normal except for a slight reaction from 20/2/32-26/2/32, probably as a result of an injection with crushed mosquitos. At the site of the injection an abscess several cm. in diameter developed. On 9/3/32 this horse received intrajugularly 5 c.c. blood from Horse 20302 (*vide*) the temperature reaction setting in on the 2nd day p.i., the animal succumbing on the 5th day p.i. from a mixed horse-sickness reaction. At the post-mortem examination the diagnosis was confirmed by the presence of a marked transudation into the subcutis and loose connective tissues, a marked hydropericard, oedema of the lungs, subendocardial haemorrhages and fatty degeneration of the liver.

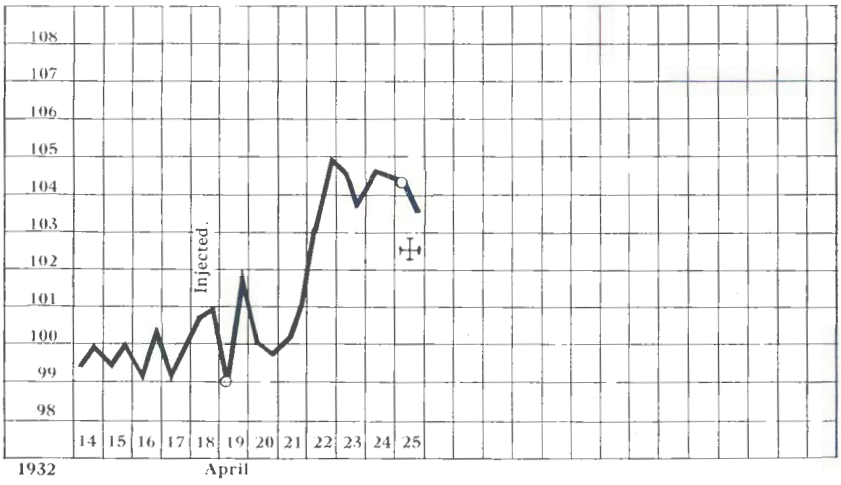
			Table 12. *Horse* 20270.		Table 13. *Horse* 20307.		Table 14. *Horse* 20319.		
Date			21/3/32.	23/3/32.	19/4/32.	25/4/32.	21/4/32.	22/4/32.	25/4/32.
Time			—	—	—	—	—	—	—
Temperature Reaction			P.I.N.	R	P.I.N.	R	N	P.I.N.	R
Hb. gm. %			19·72	12·13	16·54	17·84	16·87	19·33	16·29
Sugar mgm. %		L	82·00	86·96	—	80·65	67·57	94·36	116·28
		U	74·63	82·64	—	69·44	—	79·36	107·50
T.N. gm. %			2·962	2·458	3·312	3·060	3·326	3·578	3·051
N.P.N. mgm. %		L	23·08	24·03	27·27	25·00	31·66	30·00	26·84
		U	17·97	17·57	16·66	16·77	23·08	24·26	19·61
Coag. N. gm. N %		L	2·939	2·434	3·285	3·035	3·294	3·548	3·024
		U	2·944	2·440	3·295	3·047	3·308	3·554	3·031
Urea	mgm. N %	L	10·24	9·25	8·13	7·00	14·03	15·00	10·70
	„ U %		21·42	19·53	17·00	14·70	29·40	31·50	22·47
	mgm. N %	U	10·11	9·25	7·33	6·66	13·29	14·38	9·81
	„ U %		21·21	19·53	15·33	14·07	27·93	30·24	20·58
Total Creatinine	mgm. N %	L	2·23	2·23	1·97	1·78	1·98	1·91	1·78
	„ TC %		6·00	6·00	5·32	4·80	5·32	5·14	4·80
	mgm. N %	U	1·84	1·78	1·41	1·52	1·30	1·41	1·30
	„ TC %		4·96	4·80	3·78	4·10	3·60	3·78	3·60
Uric acid	mgm. N %	L	0·29	0·27	0·35	0·37	1·89	1·04	0·78
	„ UA %		0·88	0·87	1·05	1·10	5·66	3·18	2·34
	mgm. N %	U	0·30	0·48	0·22	0·18	0·18	0·40	0·30
	„ UA %		0·89	0·16	0·67	0·54	0·53	1·19	0·89
Amino acid mgm. N %		L	7·00	5·66	11·66	10·00	8·75	10·00	8·43
		U	4·52	3·50	3·68	3·89	4·80	4·67	4·12
R.N. mgm. N %		L	3·32	6·65	5·16	5·85	5·05	2·06	5·08
		U	1·20	2·88	4·02	4·52	3·51	3·40	4·08
Plasma			—	—	n.u.	Orange yellow	n.u.	Orange	Orange

Main Features of Analytical Data.

In the case of these three horses no "normal" values were determined before the injection of virus and to establish what is "abnormal", is therefore, somewhat difficult, particularly since the normal variations are relatively large. With the exception of a tendency towards an increase in sugar in Case XIV and figures for R.N. rather on the high side of normal, no specific alterations can be noted.

Case XIII.

Horse 20307. *Horse-sickness (Dikkop). Died* 25/4/32.
Temperature Chart XIII.



History: Horse 20307. An aged grey gelding, in poor condition. Was placed on temperature on 14/3/32 and received an intrajugular injection of 5 c.c. blood from Horse 20265 (O.virus 191st generation) on the 18th April, 1932. The temperature reaction set in on the 4th day p.i., reaching 105° within 24 hours, the animal dying on the 7th day p.i. It showed typical symptoms of Dikkop horse-sickness.

The post-mortem revealed that post-mortem changes were fairly well advanced but the gross alterations associated with Dikkop horse-sickness were present.

Case XIV.

Horse 20319.

No Temperature Chart.

A bay gelding, aged, in fair condition. Was injected for virus purposes on 22/4/32, but was too wild at the start to submit to temperature taking. On the 5th day the temperature was 105·6°. The horse was then shot. At the post-mortem there was found to be present, hydropericard, fibrinous pleuritis, chronic fibrinous peritonitis and verminosis.

(*d*) Normal Range of Values for Hb., Sugar, T.N., N.P.N., U.N., T.C.N., U.A.N., A.A.N., and Rest N.

For the purpose of compilation of the normal values, only data obtained prior to the infection with virus has been considered here. During the determinations every endeavour was made to work as accurately as possible and to check the results by means of duplicate analyses. Where the duplicates were at variance with each other, a triplicate aliquot was analysed in the majority of cases. The data recorded throughout this article are, therefore, as accurate as the limitations of the methods permitted.

Unfortunately, the number of "normal" figures I am able to submit is relatively small (40–50 only), and have been collected from animals of various breeds and ages and taken at different times of the year. My only reason for publishing them here at this stage is that they have been obtained from the same animals which were subsequently infected, which received the same diet, lived in the same environment and because a basis for evaluation is so essential for the interpretation of the pathological data collected during these researches. A few figures are also included from animals not tabulated here, but which were examined several times in succession but later were found to be immune.

Haemoglobin.—The maximum variations encountered ranged from 10·64–20·18 gm. Hb. per cent. The range of variation is more clearly demonstrated hereunder by tabulating the number of times the "Hb. gm. per cent." fell into the specified groups.

"*Hb. gm. %*."	*Occurrence.*	"*Hb. gm. %*."	*Occurrence.*
9–10	0	15–16	11
10–11	1	16–17	8
11–12	2	17–18	4
12–13	0	18–19	3
13–14	4	19–20	1
14–15	7	20–21	1

The average range is, therefore, from about 13–19 gm. Hb. per cent. with a narrower limit of 14–17 gm. Hb. per cent.

The variation in the normal Hb. content of individual animals over a given period is, as a rule, more restricted, e.g., variations are in Case IV: 13·31–17·51 gm. Hb. per cent.; in Case VI: 14·95–17·18 gm. Hb. per cent.; in Case X: 13·25–19·15 gm. Hb. per cent.; and in Case VII: 14·72–16·87 gm. Hb. per cent.

Neser (1923) drew attention to similar variations in his researches on the "Percentage volume or count of red cells" from one and the same horse over short periods, and on the basis of numerous counts in various types of horses, after a discussion of the factors involved, comes to the conclusion that:—

"(1) Moderate periods of starvation, thirst or exercise, do not influence the percentage volume of the red corpuscles of jugular blood to any appreciable extent for any length of time; (2) Food or water, after moderate periods of stravation or thirst, probably causes a slight and very temporary increase or decrease in the percentage volume in the jugular vein; (3) The mechanical state of the circulation is a very important factor influencing the percentage volume or count of the red cells in any part; a slow peripheral circulation results in concentration there and dilution in the jugular vein; (4) Variations occur in the percentage volume or count of the red cells of the blood of the same animal on different dates and are due to factors which so far have not been controlled."

Neser referred here more particularly to variations encountered in the same animal at the same time in different parts of the circulation (venous and capillary), but his results obviously also apply to differences found in the same animal from day to day (in the absence of pathological conditions). When bleeding animals at short intervals, even at the same time on each day, we cannot for a moment assume that the physiological state of the circulation is exactly the same as at the previous bleeding, and that the Hb. figure obtained is, therefore, an "absolute" index to the Hb. content and that any variations would represent an absolute increase or decrease in the total amount of Hb. in the circulation. An absolute decrease or increase under normal conditions as a result of physiological adaptation to environment (e.g., altitude) increased or decreased work, etc., would be relatively gradual. Even under normal conditions, however, such absolute changes are possible, if one remembers that erythrocyte destruction and regeneration is a continuous process and unless these two processes are extremely delicately adjusted to each other at all times, variations in the Hb. content occur, even if only to a slight extent.

Sugar.—For "laked" filtrates the maximum variation was found to be from 57·47–119·10 mgm. per cent. with 80 per cent. of the data falling within the 70–100 mgm. per cent. range: for "unlaked" filtrates the corresponding ranges are from 54·30–100 mgm. per cent. and 65–90 mgm. per cent. (70 per cent.). The following table reflects the distribution more clearly:—

"Laked"		*"Unlaked"*	
Sugar mgm. %.	*Occurrence.*	*Sugar mgm. %.*	*Occurrence.*
55 - 60	1	50– 55	2
60– 65	1	55– 60	6
65– 70	3	60– 65	1
70– 75	5	65– 70	5
75– 80	1	70– 75	6
80– 85	11	75 80	2
85– 90	3	80– 85	10
90 - 95	6	85– 90	3
95 100	4	90– 95	1
100–105	2	95–100	2
105–110	2		
110 120	2		

Although wide variations may be experienced in one and the same animal, it is the exception, the variations being rather individual in this sense, that in some animals the "mean" level is higher, in others lower.

There is a considerable difference between the blood sugar content of "laked" and "unlaked" filtrate, as obtained by this method, the differences being from 3·3–37·5 per cent. with an average of 16 per cent., the "laked" filtrate being always the higher. The average obtained by the addition of *all* the normal figures and dividing by the number of analyses is 89·80 mgm. per cent. and 74·80 mgm. per cent. for "laked" and "unlaked" filtrate, respectively.

If, as is generally assumed, the distribution of blood sugar between plasma and corpuscles is approximately equal, the only conclusion to be arrived at is that with the destruction of the cellular elements other reducing substances are liberated and these affecting the total "sugar" percentage.

Non-Protein Nitrogen.—In the case of N.P.N., the maximum variation is from 18·65 mgm. N per cent.–31·66 mgm. N per cent. for " laked " and from 13·63 mgm. N per cent.–23·08 mgm. N per cent. for " unlaked " filtrate. The following table indicates the distribution :—

Laked.

mgm. N %.	*Occurrence*	*mgm. N %.*	*Occurrence*
18–19	2	25–26	5
19–20	4	26–27	3
20–21	3	27–28	3
21–22	4	28–29	2
22–23	4	29–30	0
23–24	7	30–31	0
24–25	3	31–32	1

Unlaked.

mgm. N %.	*Occurrence*	*mgm. N %.*	*Occurrence*
13–14	2	20–21	3
14–15	4	21–22	2
15–16	5	22–23	1
16–17	5	23–24	1
17–18	5		
18–19	10		
19–20	1		

From the above it can be seen that the majority of data falls into the groups 18–28 mgm. N per cent and 14–22 mgm. N Per cent. for " laked " and " unlaked " filtrates, respectively. The average is 18·9 mgm. N per cent. and 17·3 mgm. N per cent, respectively. The percentages variation between " laked " and " unlaked " is from 14·2–34·6 per cent. with an average of 25·1 per cent. For the N.P.N. an individual variation is also noticeable, i.e., animals are encountered who have either a relatively high or a relatively low normal N.P.N. level, e.g., c.f., Case IV with 18·64–24·04 mgm N per cent., Case V with 24·4–27·8 mgm. N per cent., Case VI with 19·4–22·9 mgm N per cent., and Case X with 22-26 mgm. N per cent.

Urea.—The normal urea nitrogen content yields maximum variations of from 4·7–14 mgm. N per cent (9·9–29·4 mgm. Urea per cent.), and from 4·4–13·3 mgm. N per cent. (9·3–27·9 mgm. Urea percent.), respectively, for " laked " and " unlaked " filtrates. To indicate the distribution more accurately, the following table is attached, but owing to the small variations between the urea nitrogen content in the two filtrates, all the analytical data has been incorporated in the one column.

mgm. U.N. %.	*Occurrence*	*mgm. U.N. %.*	*Occurrence*
4–5	2	10–11	14
5–6	4	11–12	10
6–7	15	12–13	6
7–8	8	13–14	3
8–9	8	14–15	1
9–10	13		

It will be noted that the majority of analytical figures lie between 6–13 mgm. N per cent. (12·6–27·3 mgm. Urea per cent.) with the maximum at ± 10 mgm. N per cent. (23·1 mgm. Urea per cent.). If the average of all the "laked" and "unlaked" is calculated, the figures 9·3 mgm N per cent. and 8·8 mgm N per cent., respectively, are obtained. The variations encountered are also more or less individual, i.e., each animal within the prescribed limits has its own mean "U.N." content, which is either relatively low, c.f., Case IV with 6·24–9·87 mgm N per cent.: Case VI with 4·7–7·3 mgm. N per cent.; and Case VIII with 9·25–11·58 mgm. N per cent.

Totale Creatinine Nitrogen.—The normal range is from 1·52–2·80 mgm. T.C.N. per cent. (4·1–7·6 mgm T.C. per cent.) and 1–2·23 mgm. T.C.N. per cent. (3·0 6 mgm. T.C. per cent.) with an average of 2·1 mgm T.C.N. per cent. and 1·8 mgm. T.C.N. per cent. for "laked" and "unlaked" filtrates, respectively. The following table gives a more accurate conception of the range of distribution:—

"*Laked.*"		"*Unlaked.*"	
mgm. T.C.N. %.	*Occurrence*	*mgm. T.C.N. %.*	*Occurrence*
1·50 2·00	12	1·00 1·50	9
2·00–2·50	27	1·50–2·00	17
2·50–3·00	2	2·00–2·50	14

From this it is evident that the mean T.C.N. concentration lies from 2·00–2·50 mgm. T.C.N. per cent. (5·4–6·8 mgm. T.C. per cent.). The percentage differences between the "laked" and "unlaked" concentrations vary from 0–35 per cent. with an average of 16 per cent.

Although variations exist the relatively most constant values of any of the nitrogenous fractions sought for were found in this group, e.g., in Case IV, the date is 2·42, 2·42, 2·42 and 2·01 and 2·23 mgm. for "laked," and 2·23, 2·23, 2·23, 2·23 and 2·10 mgm. "unlaked."

As was shown to be the case in the previously discussed constituents, each individual has its own particular level, e.g., Case VI: 2·06, 1·91, 1·67, 1·52, 1·91, 1·98 for "laked," and Case IV: 2·42, 2·42, 2·42, 2·01, 2·23 for "laked," respectively.

Uric Acid.—The normal variations encountered ranged from 0·23–0·82 mgm. U.A.N. per cent. (0·70–2·5 mgm. U.A. per cent.) and 0·13–0·53 mgm. U.A.N. per cent. (0·40 1·6 mgm. U.A. per cent.) in "laked" and "unlaked" filtrate respectively. The average figures are 0·41 mgm. T.C.N. per cent. and 0·25 mgm. T.C.N. per cent.

The following table indicates the number of times each particular concentration was met with:—

"*Laked.*"		"*Unlaked.*"	
U.A.N. mgm. %.	*Occurrence*	*U.A.N. mgm. %.*	*Occurrence*
0·20–0·30	12	0–0·10	0
0·30–0·40	8	0·10–0·20	15
0·40–0·50	12	0·20–0·30	12
0·50–0·60	3	0·30–0·40	6
0·60–0·70	2	0·40–0·50	3
0·70–0·80	2	0·50–0·55	1
0·80–0·82	1	0·50–0·60	0

From this the majority of figures are seen to range from 0·20–0·50 mgm. U.A.N. per cent. and from 0·10–0·40 mgm. U.A.N. per cent. The differences between the "laked" and "unlaked" range from 0 per cent. to 70 per cent., with an average of 36·5 mgm. per cent., the "laked" content being the higher. Each individual has its own particular level. This clearly emerges from Case VIII, where the average U.A.N. per cent. is 0·25 mgm. and Case X where it is 0·38 mgm. and Case VI where is is 0·48 mgm. N per cent. and correspondingly less in the "unlaked" filtrates.

Amino Acids.—This fraction ranges from 5·38–10·80 mgm. N per cent. and from 3·50–5·83 mgm. N per cent. with an average of 7·90 mgm. N per cent. for "laked" and 4·43 mgm. N per cent. for "unlaked." This fraction appears to be the only nitrogenous fraction differing from the others in so far as no "individual" level appears to exist.

Rest Nitrogen.—The R.N. (or "undetermined" nitrogen) here represents that portion of the N which remains after the U.N., U.A.N., T.C.N., and A.A.N. have been subtracted from the N.P.N. It is obtained by calculation and, therefore, its accuracy depends on the accuracy of each determined N fraction.

In the case of the horse the maximum variations range from 1–6·66 mgm. N per cent. and from 0·20–4·44 mgm. N per cent. in "laked" and "unlaked" filtrates, respectively. The percentage differences range from ± 10 per cent. to ± 60 per cent., with an average of 43·3 per cent. between "laked" and "unlaked" respectively.

The "average" R.N., taking all the normal data into consideration, is 3·70 mgm. N per cent. and 2·11 mgm. N percent., respectivcly, the "laked" being in the vast majority of cases higher.

Total Nitrogen.—The T.N. showed variations from 2·7 gm. N per cent.—3·6 gm. N per cent. with an average of—3 gm. N per cent., depending partly on the Hb. content. There is, therefore, also an individual level for T.N. which, under relatively constant conditions, remains fairly constant for each particular animal.

(*e*) General Summary of Range of Values during Horse-sickness.

This summary of the composition of blood gives the essential variations found in fourteen cases of horse-sickness of which six cases were of the Dikkop form, three of the Dunkop form, three of the mixed form, and one animal was shot before pathognomic symptoms could be noted, i.e., out of fourteen cases only one recoverd. It is, therefore, evident that on the score of virulency, nothing remained to be desired.

Analyses were performed frequently: during the reaction every 48 hours, frequently every 24 hours, till death supervened. Considering the constituents one by one, the following main features were observed during the course of the reactions.

Haemoglobin.—This showed no characteristic differences from normal. In some cases (Ix, X, XIII, XIV) the Hb. content remained unchanged throughout; in others a slight decrease could be seen (III, XI, XII) in others, on the other hand, a slight increase (IV and VI). Some few cases showed a slight drop followed by an increase (I and VIII); others again the reverse (II and VI). The highest Hb. content appeared in two horses bled one and four hours,

respectively, before death. (23 and 25 gm. Hb. per cent.). In *all* the other animals the variations *were within the normal range* and may, therefore, have been coincidental, although I believe that they are associated in some way with the horse-sickness reaction.

This disease is not in the true sense a blood disease such as one would regard anaplasmosis or piroplasmosis, and severe anaemias, therefore, do not occur unless, of course, complicated with infectious anaemia or biliary fever (*P. caballi* and *N. equi* infection) as a result of breakdowns of immunity. There would, however, appear to be a slight destruction of erythrocytes in the majority of cases as a change in the colour of the serum to an orange-yellow—indicative of icterus—is quite common. Furthermore, other factors would tend to upset the Hb. level such as e.g., the water retention so frequently found in fevers. This would tend relatively to decrease the Hb. content. The tremendous transudation and exudation into the lungs, pleural cavities and subcutis would lead to a concentration of the blood, even if only temporary, and thus to an increase of the Hb. index.

Sugar.—In eight cases (I, II, III, IV, V, X, XI, XIV) an increase in the blood sugar level took place; the maximum figure obtained being 161·3 mgm. per cent. (Case V) bled an hour before death. In the remaining cases the increases are only slightly above the upper level of normal, or even below it. The increase in such cases was only relative in comparison with the normal or preinfection content. In the remaining six cases no changes were detectable (VI, VII, VIII, XI, XII, XIII).

Total Nitrogen.—No specific alterations occur, minor increases and decreases being associated with the corresponding changes in the Hb. content.

Urea Nitrogen.—In most cases there is a tendency towards a slight decrease.

Non-Protein Nitrogen.—Shows no distinctive alterations.

Uric Acid Nitrogen.—Shows no distinctive alterations.

Amino Acid Nitrogen.—Shows no distinctive alterations.

Total Creatinine Nitrogen.—Shows no distinctive alterations.

Rest Nitrogen.—With the exception of Case VII where it is increased, no definite variations occur. Case V forms an exception in so far as the above statements are concerned. All the constituents on the last day (four hours before death) were increased considerably. This would appear to be due to inspissation of the blood and not due to specific changes associated with horse-sickness, since all the constituents are practically equally affected.

(*f*) Conclusion.

The changes in the composition of the blood encountered in horse-sickness are practically nil, and considering the severe symptoms and the striking post-mortem findings, the negative results are surprising. It is true that in most cases the disease ran a very acute course, and it may be urged that, therefore, the time period for a difference in composition to develop was too short. This may partially explain the virtually negative findings, but it should be pointed out that in the one case of recovery no more marked alterations occurred than in the others. One case is obviously insufficient to permit of any definite conclusions being drawn, and it is the intention to undertake analyses in "recovery cases" as apportunities occur. Very scant data exists of chemico-pathological blood examination in horses during other diseases, but conceivably

an explanation for the relative stability of the composition of the blood finds a physiological explanation in the light of the peculiar development of the present day horse. Amongst all our domestic animals it is the fastest and most active, capable of heavy, long continued work, or spurts of intensive activity (race horses). For this a highly developed efficient circulation is a *sine qua non* and the maintenance of an effective mechanism for maintining the normal composition of the blood and the efficient removal of waste products is essential. Further chemical researches into the composition of the blood of the horse during health and disease are necessary before any explanation advanced becomes of more than argumentative interest.

Attention was drawn to the existence of "individual" levels for most of the normal nitrogenous fractions such as N.P.N., U.N., T.C.N., etc. These variations may be associated with such factors as condition, "fitness," stamina, temperament, age, sex, normal functional capacity of the kidneys, basic tissue, metabolism, etc., or undiagnosed chronic conditions, but without a greater mass of normal data collected for the special purpose of determining the causes of these variations, no explanation based on experimental evidence is possible.

This line of investigation would in my opinion, be of interest, enabling one possibly even to pick out a potential winner—or loser!—out of a bunch of race horses or a good milker-to-be out of a herd of yearling heifers, on the basis of accurate chemical blood examinations alone!

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