placed at her disposal by the Hospital for Sick Children, Toronto, has found the incidence of intestinal parasites in Toronto children to be much higher than is generally appreciated.

Mrs. H. T. Malloy, working in the University Clinic, Royal Victoria Hospital, Montreal, has investigated hereditary jaundice in rats and has found that it does not depend upon enhanced hemolysis but rather in the inability of parenchyma liver cells to deal properly with blood bilirubin, *i.e.*, the hereditary factor concerns parenchyma liver cells rather than haemopoietic tissue.

Dr. D. G. H. Macdonald, working in the Department of Physiological Hygiene, University of Toronto, reports the results of his study with regard to vitamin B deficiency and slow heart rate. It was found that this latter condition was due specifically to lack of vitamin B_1, but that it was not alleviated by B_1 alone; adequate food intake was needed as well.

Dr. D. W. G. Murray and Dr. R. G. MacKenzie, Department of Surgery, University of Toronto, report results on further experimental and clinical use of heparin. Heparin is shown to facilitate blood-vessel surgery by preventing thrombosis. Its ability to prevent thrombosis in thrombophlebitis, as well as its ability to prevent further thrombosis and embolism in cases where it has already occurred was also established.

Dr. B. Rose, University Clinic, Royal Victoria Hospital, Montreal, reports the results of several studies on histamine. The kidney was found to take up most of the histamine injected into the rat's blood stream. Kidney, however, was found to be devoid of histaminase. Adrenalectomized rats were unable to inactivate histamine. Injections of cortin restored their normal ability to inactivate it.

Mr. E. A. Ryan, working in the Department of Biochemistry, University of Toronto, reports that previously used methods have not been productive in allowing him to isolate and identify a new compound in male urine. New methods have, however, been utilized which promise to be of considerable help in this and similar researches, and already there is indication that a new ketone has been found.

Dr. M. A. Sergeyeva, working in the Department of Physiology, McGill University, Montreal, reports that definite changes occur in the islet cells of the pancreas when the autonomic nerves supplying that organ are cut or stimulated experimentally. She has furthermore found that, under certain experimental procedures of this type, numbers of cells displaying characteristics of both islet and acinar cells appear.

Drs. R. W. I. Urquhart and D. L. Selby, working in the Department of Pathological Chemistry, University of Toronto, report further progress with their study of experimental nephrosis. They have tested the effects of a standard damage to a more or less specific part of the tubule of one kidney with regard to the elimination of many ions in addition to the chlorine ion.

Dr. P. G. Weil, working in the University Clinic, Royal Victoria Hospital, Montreal, has found that normal individuals do not excrete cortin. It was found, however, that cortin was excreted (1) in certain disease conditions and (2) following operations where its excretion reached a peak in four or five days. Studies on the relationship of cortin to surgical shock are in progress.

V. E. HENDERSON
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SPECIAL ARTICLES

THE EFFECT OF THIOL COMPOUNDS ON GONADOTROPHINS[1]

CYSTINE and cysteine in protein molecules have hitherto been regarded as existing in two definite forms —one in which the sulfur-containing groups give the reactions for sulfhydryl or disulfide compounds as these are given by the free amino acids and another form in which these reactions are not given. Denaturation is known to cause the transformation of many non-reactive to reactive[2] groups.

It has only recently been recognized that –SH groups of intermediate reactivity occur in both native and denatured proteins. It has also been shown that –SH groups exist which will react with some and not with other reagents.[3, 4, 5, 6] Results here reported indicate that –S–S– bonds of intermediate reactivity may exist in native proteins. This study was done on highly physiologically active proteins—gonadotrophins—which, however, are not chemically pure; analytical data concerning the state of reduction of such mixtures

[1] Aided by grants from the Research Board of the University of California; from the Rockefeller Foundation of New York and from Parke, Davis and Company of Detroit, Michigan. Assistance was rendered by the Federal Works Progress Administration, Project OP 65-1-08-62, Unit A-5.

[2] The terms "reactive" and "reactivity" are used here only to indicate whether or not the reactions typical for a certain chemical group are given.

[3] J. P. Greenstein, *Jour. Biol. Chem.*, 125: 501, 1938.

[4] M. L. Anson, *Jour. Gen. Physiol.*, 23: 239, 1939.

[5] A. K. Balls and H. Lineweaver, *Nature*, 144: 513, 1939.

[6] M. L. Anson, *Jour. Gen. Physiol.*, 23: 321, 1940.

of proteins would therefore appear to be of little value.[7] As has previously been reported, pituitary gonadotrophins are inactivated by cysteine.[8] In the present study the degree of inactivation was therefore used to estimate the extent to which the reaction between cysteine and the protein had proceeded. As Table I shows, solutions of 1 mg per cc of various gonadotrophins were almost completely inactivated when treated with a 40-fold amount of cysteine for 2 days at room temperature and pH 7.8; while none of these gonadotrophins was inactivated at concentrations below 0.1 mg per cc under otherwise identical conditions. Nevertheless, if such dilute mixtures of gonadotrophins with cysteine or thioglycolic acid were permitted to stand for longer periods of time or at higher temperatures, marked inactivation resulted. It must be noted that no inactivations were observed in control solutions kept under the same conditions but without the reducing agent. It has been observed that cysteine treatment in 40 per cent. urea solution causes a very much more rapid inactivation of gonadotrophins than that in aqueous solution under otherwise identical conditions.[9] This observation is in good agreement with the established fact that the denaturation of many proteins causes an increase in the reactivity of –SH, –S–S– and other groups.

From these experiments it appears that groups which are essential for the activity of all gonadotrophins thus far studied are affected by these thiol compounds. Since no protein groups besides –S–S– bonds have been shown to react with such compounds, the assumption appears justified that the reduction of certain of these bonds causes the observed inactivation of gonadotrophins. The rate of this reaction is unusually slow, and under the specified conditions the attainment of equilibrium within 48 hours depends on the concentration of the reacting substances. It must be noted that our previous conclusion[8] that as regards their reaction with thiol compounds, a difference exists between the gonadotrophins of pituitary as contrasted with those of chorionic origin can not now be maintained. The former conclusion was drawn before full realization of the importance of protein concentration in these reactions. It may be repeated that thiol com-

[7] Such data are being collected in a similar study of a pure protein hormone (mammotrophin).

[8] H. Fraenkel-Conrat, M. E. Simpson and H. M. Evans, *Jour. Biol. Chem.*, 130: 243, 1939.

[9] The commercial detergent, Duponol PC, which was observed by Anson (footnote 4) to resemble urea in its denaturing effect on proteins, did not appreciably increase the rate of cysteine inactivation of these hormones.

TABLE I

Gonadotrophine	Reagent*	Reaction: Protein mg/cc	Reaction: Time days	Reaction: °C.	Solvent	MED† mg
Pituitary FSH (IVF20B)	.....	0.025	2	22	phosphate buffer (pH 7.8)	0.02
" " "	cysteine	0.5	2	22	" " "	0.15
" " "	cysteine	0.05	2	22	" " "	0.03
" " (IVF28A)	.....	...	..	..	dialysed solution	0.015
" " "	.....	0.05	5	22	phosphate buffer (pH 7.8)	< 0.03
" " "	cysteine	1.0	5	22	" " "	> 0.25
" " "	cysteine	0.05	5	22	" " "	0.05
" " "	.....	0.05	2	40	" " "	< 0.03
" " "	cysteine	0.05	2	40	" " "	> 0.15
" " (IVF28B)	.....	0.05	2	22	40 per cent. urea (pH 7.8)	0.015*
" " "	thiogly-colic acid	0.05	2	22	" " " " "	> 0.05*
Chorionic (Antuitrin S)	.....					0.005
" "	cysteine	1.0	2	22	phosphate buffer (pH 7.8)	> 0.1
" "	cysteine	0.05	2	22	" " "	0.007
" "	.....	0.05	1	22	40 per cent. urea (pH 7.8)	0.015
" "	cysteine	0.05	1	22	" " " " "	> 0.09
Teratoma testis urine	.....	1.0	2	22	phosphate buffer (pH 7.8)	0.0015
" " "	cysteine	1.0	2	22	" " "	0.1
" " "	cysteine	0.1	2	22	" " "	0.0015
" " "	cysteine	0.05	2	22	" " "	< 0.0025
Pregnant mare serum (Gonadin)	.....	0.25	2	22	phosphate buffer (pH 7.8)	0.015
" " "	cysteine	1.0	2	22	" " "	0.25
" " "	cysteine	0.05	2	22	" " "	< 0.025
" " "	.....	0.05	9	22	" " "	< 0.025
" " "	cysteine	0.05	9	22	" " "	> 0.15
" " "	.....	0.1	1	0	" " "	0.015
" " "	thiogly-colic acid	0.1	1	0	" " "	0.015
" " "	thiogly-colic acid	0.1	1	22	" " "	0.05
" " "	.....	0.1	1	40	" " "	0.015
" " "	thiogly-colic acid	0.1	1	40	" " "	0.3

* Of the reducing agents a 40 fold of the protein is used throughout.

† With the exception of those marked with an asterisk which were done in hypophysectomized rats all standardizations were done in normal immature rats. 1 unit of IV F 20B was contained in 0.004 mg of IV F 28A and IV F 28B in 0.006 mg, as measured by our routine test for FSH fractions in hypophysectomized rats or by augmentation with chorionic gonadotrophin.

pounds decrease or destroy the potency of all gonadotrophins thus far investigated.

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THE SYNTHESIS OF PHOSPHOPYRUVIC ACID ON OXIDATION OF LACTIC ACID

THE formation of pyruvic acid from phosphopyruvic acid, and its further conversion into lactic acid, are well-studied stages of the anaerobic decomposition of carbohydrates, whereas the reversal of these reactions has not yet been fully investigated.*

It is known that lactic acid on oxidation yields pyruvic acid, whose further course of aerobic decomposition is known. We have established that pyruvic acid produced by the oxidation of lactic acid can be phosphorylated, giving rise to phosphopyruvic acid. The synthesis of phosphopyruvic acid has been effected in minced muscle tissue by adding to it sodium lactate with a good supply of oxygen. Along with the synthesis of phosphopyruvic acid there is a decrease in the amount of inorganic phosphate. The presence of phosphopyruvic acid was recognized by its instantaneous splitting in the presence of mercury ions, and also from its splitting by iodine in alkaline medium, giving rise to iodoform and inorganic phosphoric acid. As an example we may report the results of one of our experiments.

The muscle of a cat was minced by means of scissors. Out of this homogeneous mass the following samples were taken:

Sample A: 20 g of tissue incubated for 90 min. at 40° C. with a good supply of oxygen in 50 cc of 2 per cent. $NaHCO_3$ prepared from m/10 sodium lactate + 10 cc H_2O + 40 mg KH_2PO_4 (0.45 mg per 1 g of tissue).

Sample B: Prepared as sample A + 0.1 g of NaF.

Sample C: 20 g of tissue incubated for 90 min. at 40° C. in 2 per cent. $NaHCO_3$.

Sample D: Control sample, without incubation. 20 g of tissue were placed in a 7 per cent. solution of trichloracetic acid.

After the incubation the proteins were precipitated with 20 cc of 25 per cent. trichloracetic acid. Filtration and analyses were carried out after 20 hours standing in the refrigerator.

Phosphopyruvic acid is absent in samples C and D. It must therefore have been absent from the tissue in its preformed state. Nor did it accumulate during the incubation without the addition of sodium lactate and without oxygen supply.

TABLE 1

IN MG OF H_3PO_4 – P PER 1 G OF MUSCLE TISSUE

Samples	H_3PO_4 – P after hydrolysis in nHCL at 100° C.					Decrease of the inorganic H_3PO_4 – P	Amount of H_3PO_4–P formed on hydrolysis in nNaOH within 30′ (triose-phosphate P′)	Amount of H_3PO_4–P formed on treatment of the protein-free-filtrate with 2nNaOH and I (phosphopyruvic acid – P + triose-phosphate – P)
	0′	7′	30′	60′	90′			
A	0.56	0.77	0.93	0.98	0.98	0.89	0.02	0.20
B	0.27	0.32	0.38	0.41	0.41	1.18	0.05	0.15
C	1.57	1.63	1.63	1.71	1.71	...	0	0
D	1.00	1.33	1.38	1.38	1.38	...	0	0

Sample A shows a considerable content of phosphopyruvic acid. The amount of acid actually formed in this sample is much larger than what may be inferred from the figure tabulated, as phosphopyruvic acid is in enzymatic equilibrium with phosphoglyceric acid (according to Meyerhof and Lohmann's[1] data, equilibrium in enzymic solutions at 20° C. is established at 29 per cent. phosphopyruvic acid and 71 per cent. phosphoglyceric acid). The decrease of the amount of the inorganic phosphate, and the accumulation of phosphate stable toward hydrolysis in nHCL, indicate that phosphoglyceric acid was formed along with phosphopyruvic acid.

The formation of phosphopyruvic acid in sample B is particularly conclusive. It is known that NaF blocks the reaction: Phosphoglyceric acid $\leftrightarrows$ phosphopyruvic acid. This rules out the possibility of phosphopyruvic acid being a product of the decomposition of glycogen under our experimental conditions.

In 1924 Embden and his co-workers[2] observed the decrease of inorganic phosphate on incubating a minced muscle tissue in the presence of lactate. They believed the anions of the lactic acid to possess a specific power for inducing the synthesis of hexosephosphate ("lactacidogen") in muscle. The incorrectness of this early opinion was proved by one of us (D.F.),[3] who found that a muscle tissue yields on incubation in lactate a compound which hydrolyses in nHCL in 30 min. and does not reduce $K_3Fe(CN)_6$. The data now obtained have elucidated the nature of the phosphoric compound thus produced by a muscle tissue on incubation in lactate.

There are reasons to think that lactate is not the only substrate for the synthesis of phosphopyruvic acid. Evidence of this is afforded by the investigations of Kalckar,[4] who observed the formation of phosphopyruvic acid in renal tissue on oxidation with malic acid.

* Editor's note: Compare, however, the papers of Meyerhof, Ohlmeyer, Gentner and Maier-Leibnitz (*Bioch. Z.*, 298: 396 (1938) and Green, Needham and Dewan, *Bioch. J.*, 31: 2327 (1937).

[1] Meyerhof and Lohmann, *Biochem. Z.*, 273: 60, 1935.
[2] Embden and co-workers, *Z. Physiol. Chem.*, 143, 1924.
[3] Ferdman, *Z. Physiol. Chem.*, 187: 160, 1930.
[4] Kalckar, *Biochem. Jour.*, 33: 631, 1939.