were made with the various fractions of the ragweed extracts. Patients who suffered from allergic disease but who were not clinically sensitive to ragweed pollens failed to show positive reactions to scratch tests with the various fractions. Patients who were clinically sensitive to ragweed pollen showed significantly larger skin reactions with the more highly purified extracts than with the less highly purified extracts or with the untreated extracts.

Summary. 1. Fractionation of aqueous ragweed pollen extracts with water-miscible organic liquids produces purification and concentration of the allergens of these extracts. 2. Fractionation of extracts, purified by the above treatment, with high concentrations of sulphate salts produces further purification and concentration of the allergenic factors.

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Changes in Blood Ketone Acids During Artificial Fever.

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Fever, whether infectious or artificial in origin, increases the metabolic rate and consequently influences carbohydrate metabolism. The initial effect is an increased utilization of carbohydrate in the tissues, resulting in an accelerated withdrawal of glucose from the blood. As a means of compensating for the drain on blood sugar, a rise in the rate of hepatic glycogenolysis takes place, and this in turn leads in the postabsorptive state (or when glucose is absorbed at a lower rate than it is removed from the blood) to a depletion of the glycogen stores of the liver.

Observations on patients and on laboratory animals, undergoing treatment with hyperthermia, clearly show the process in its several phases. In a previous communication[1] we pointed out the fact that the hyperglycemia that occurs during fever is due to excessive hepatic glycogenolysis, and not to blood concentration, as several workers believed. In accord with this view, von Haam[2] and Gunderson and

1 Kirstein, M. B., and Bromberg, L., *J. Lab. and Clin. Med.*, 1939, **25**, 7.

2 Von Haam, E., Changes in the parenchymatous organs and blood vessels produced by artificially induced fever. Presented at conference on fever therapy, under auspices of the Kettering Foundation, St. Louis, Mo., Nov. 11, 1938.

Loseke[3] found a marked depletion of the glycogen stores in the livers of laboratory animals that were subjected to prolonged and severe hyperthermia.

Since the production of ketone bodies is intimately connected with carbohydrate metabolism, it seemed of interest to investigate possible changes in ketonemia during artificial fever. We studied 6 patients, 3 males and 3 females; 5 of these were afflicted with syphilis of the central nervous system, the sixth, an 11-year-old girl, had Sydenham's chorea. By performing glucose tolerance tests on the patients, we ascertained that their carbohydrate metabolism was normal.

In every treatment fever was maintained for 4 hours at 40 to 41° (rectally measured). Prior to and between treatments the patients received unrestricted diets; on the morning of treatment they were given 200 cc of orange juice, one cup of coffee with sugar and one slice of toast (about 45 g of carbohydrates). During hyperthermia they drank saline in quantities sufficient to forestall dehydration.

The total ketone bodies in the blood were determined at the onset and at the termination of fever. The analytical method employed was Hubbard's micromethod[4] with some unessential modifications. The maximum error in recovering known quantities of ketone bodies, and in duplication of results by this technic, is less than 10%. The figures, recorded in Table I, show that 4 hours of fever caused a significant rise in ketonemia in all of our subjects; the increase varied in 5 cases from 80 to 140%, and in the sixth case it rose to 14-fold of the initial level.

This phenomenon can be readily understood, and even anticipated, if one postulates as satisfactorily proved 2 observations of Embden, *et al.*, first, that the liver is the sole source of the ketone bodies

TABLE I.
Effect of Artificial Fever upon Ketonemia.

Patient	Sex	Age	Ketone bodies in blood, expressed as β-hydroxybutyric acid		
			Before treatment mg %	At end of treatment mg %	Increase %
P.L.	F	28	1.08	2.34	116
N.W.	F	46	0.96	1.73	80
R.C.	M	32	1.10	2.70	145
F.B.	M	26	1.04	1.87	80
M.B.	F	11	0.95	1.93	103
E.E.	M	33	0.47	6.60	1300

[3] Gunderson, M. F., and Loseke, L. L., Depletion of liver glycogen under experimental fever induction in rabbits. Presented at conference on fever therapy, under auspices of the Kettering Foundation, St. Louis, Mo., Nov. 11, 1938.

[4] Hubbard, R. S., *J. Biol. Chem.*, 1921, **49**, 375.

that occur in blood and urine,[5] and second, that an inverse relationship obtains between the glycogen content of the liver and the rate of formation of ketone bodies.[6]

The discovery of these facts grew out of perfusion studies of liver and other organs by Embden and his collaborators. Subsequently, numerous other workers, employing improved analytical technic and a wide variety of experimental approaches, concurred without a single exception in Embden's conclusions.[7-13]

Pursuing this concept, it should be possible to prevent, or at least to mitigate, the ketogenic effect of fever by stocking the liver with substantial amounts of glycogen directly before or during fever. Subject E. E. was selected for a series of experiments to probe the validity of the theory; 3 observations were made on him at weekly intervals. First he was treated in the postabsorptive state, 14 hours after his last meal. As may be seen in Table II, the ketone content of his blood rose to nearly 14-fold of the initial level. One week later the treatment was repeated; this time the patient was given breakfast and, in addition, 100 g of glucose intravenously. The injection was started 5 minutes after fever had begun, and was continued for 1 hour and 40 minutes. Hyperthermia was terminated, and a blood sample was withdrawn for analysis, 2 hours and 15 minutes after completion of the glucose infusion.

In this experiment the patient received a total of 145 g of glucose, a substantial portion of which undoubtedly was added to the glycogen stores of the liver. The fever increased the metabolism by about 30% above the normal rate,[14] equivalent to approximately 100 calories for the entire 4-hour period. Even if it is assumed that all of the 100 calories was derived exclusively from glucose, and that the corresponding 25 g of glucose required were derived altogether from hepatic glycogen, the glycogen content of the liver still could scarcely have been lower after than before the fever period. In other words, there is no justification for the assumption that the glycogen stores of the liver were depleted during the treatment. Yet, as may be

5 Embden, G., and Kalberlah, F., *Beitr. z. chem. Physiol. u. Path.*, 1906, **8**, 121.

6 Embden, G., and Wirth, J., *Biochem. Z.*, 1910, **27**, 1.

7 Burn, J. H., and Marks, H. P., *J. Physiol.*, 1926, **61**, 497.

8 Raper, H. S., and Smith, E. C., *J. Physiol.*, 1926, **62**, 17.

9 Chaikoff, I. L., *J. Biol. Chem.*, 1927, **74**, 203.

10 Edson, N. L., *Biochem. J.*, 1936, **30**, 1862.

11 Mirsky, I. A., Heiman, J. D., and Broh-Kahn, R. H., *Am. J. Physiol.*, 1937, **118**, 290.

12 Blixenkrone-Moeller, N., *Z. f. physiol. Chem.*, 1938, **252**, 117.

13 Harrison, H. C., and Long, C. N. H., *J. Biol. Chem.*, 1940, **133**, 209.

14 Simpson, W. M., *J. A. M. A.*, 1936, **106**, 246.

TABLE II.
Effect upon Ketonemia of Glucose Injection During Fever Treatment.
These observations were made on patient E.E.

Ketone bodies in blood		Blood sugar		
Before treatment mg %	At end of treatment mg %	Before treatment mg %	At end of treatment mg %	Food intake
				Experiment I
0.53	7.30	69	79	No breakfast, no glucose.
				Experiment II
0.60	3.06	90	66	Breakfast, plus 100 g glucose injected over period of 100 min.
				Experiment III
0.62	0.47	113	162	Breakfast, plus 100 g glucose injected over period of 205 min.

seen in Table II, ketonemia under these conditions has risen to about 5-fold of the initial level. This increase is considerable, even though not quite half as great as it was when the patient received no carbohydrates.

This rise in ketonemia despite the administration of carbohydrate can be explained as follows: During the injection of glucose the blood sugar level was undoubtedly above the postabsorptive level, a condition conducive to glycogen deposition in the liver. Data in hand from other experiments in this laboratory show that ketonemia during such periods declines below the postabsorptive level. Soon after completion of the injection, however, the glycemic level must have rapidly declined in this experiment, dropping below the postabsorptive level and, as may be seen in Table II, remaining low to the end of the treatment. This hypoglycemic interval induced an increase in the rate of hepatic glycogenolysis, and concurrently ketone formation was substantially accelerated. Thus it may be inferred from this experiment that an abnormal increase in the rate of hepatic glycogenolysis *per se* may increase the rate of ketone formation, without the depletion of the glycogen stores of the liver.

The validity of this assumption was tested in a third experiment on the same patient. This differed from the preceding experiment only in one respect, namely, that the injection of the glucose was prolonged, extending over a period of 3 hours and 15 minutes; it was begun 20 minutes after the onset of the fever, and completed 15 minutes before the termination of the treatment. The purpose of this procedure was the production and maintenance of hyperglycemia throughout the fever, designed to compensate for the augmented glucose requirement of the tissues, without a call at any time for an increase in the rate of hepatic glycogenolysis. The data in Table II show that we succeeded in maintaining hyperglycemia to the

end of the experiment; concurrently, it may be noted, no rise in the rate of ketone formation has taken place. On the contrary, there was a measurable drop in the ketonemic level.

May it be reiterated that the amounts of intravenously supplied glucose were identical in Experiments II and III (Table II); only the duration of the injection differed. In Experiment II, because of a shorter duration, hypoglycemia was allowed to develop during the latter part of the fever, a factor that invariably increases the rate of hepatic glycogenolysis,[15] while in Experiment III hypoglycemia was avoided by a safe margin throughout the entire experiment, so that the liver had not to be pressed into service to provide for the augmented carbohydrate need of the extrahepatic tissues.

Summary. Artificial hyperthermia in man increases ketosis. This, according to available evidence, is due to an increased rate of ketogenesis in the liver. Our observations on human subjects are in accord with the findings of previous investigators, who demonstrated on perfused liver, on liver slices and on living animals, that an inverse relationship exists between the glycogen content of the liver and the rate of ketone formation. The data presented indicate, moreover, that an increased rate of hepatic glycogenolysis *per se* suffices to increase ketosis, without any appreciable diminution of the glycogen reserves of the liver. An increased ketonemia in artificial fever can be entirely forestalled by the continuous injection of glucose so as to ensure the maintenance of blood sugar at hyperglycemic levels, a condition which enhances glycogen deposition in the liver and at the same time prevents an increase in the rate of glycogenolysis.

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Changes in Ketonemia and Ketenuria During Hypoglycemia.

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Artificial fever, as we reported, causes a substantial rise in ketonemia. Our experimental data indicated that an increase in the rate of hepatic glycogenolysis in itself suffices to accelerate the formation

[15] Somogyi, M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 51.