Again, this was confirmed by histological examination of the lungs. The alveolar and perivascular transudate was predominantly cellular and therefore differed from that in the acute edema group. Frank hemorrhage was the rule, and transudates, if present, were generally sprinkled with erythrocytes. Since hemorrhage occurred in both groups, it seems not unlikely that it is the initial event. Transudation, when it occurs, may be a secondary event, depending on the degree of respiratory obstruction and capillary asphyxia induced by hemorrhage. This hypothesis requires further study.

The data on the hyperthermic rats were collected to test the hypothesis that fever, induced by the hypothalamic lesions, causes the pulmonary changes. Table 1 contains data on 13 rats with postoperative records of elevated body temperatures ranging from 40.6° to 42.7° C; 9 of these animals died, apparently as a result of the fever. In addition, 10 animals were heated by diathermy to body temperatures of 40°–44.2° C; 7 rats died. Although the lungs of these rats showed mild, uniform discoloration, they were clearly distinguishable from those of the edematous animals. Histologically, the lungs appeared normal save for some congestion; the hemorrhage and transudate characteristic of the edematous groups were conspicuously absent. The lung weights of the hyperthermic animals were only slightly elevated over those of the control animals (Table 1). On the other hand, severe edema and hemorrhage occurred in 3 rats in which the postoperative body temperatures never exceeded 38.4° C, 37.3° C, and 38.0° C, respectively. Thus, hyperthermia is not essential to the development of pulmonary edema and hemorrhage following hypothalamic lesions.

To identify the hypothalamic region, destruction of which caused the lung edema and hemorrhage, the brains of 30 of the 33 edematous animals were sectioned serially. In addition, serial sections were cut from 26 brains of the operation control group. These brains were selected because macroscopic examination suggested that the lesions bordered the critical region. In 26 of the edematous animals there was bilateral damage to the region just overlying the rostral half of the optic chiasm. The center of the lesions was usually a millimeter from the midline and dorsal to the rostral third of the chiasm. The lesions thus occupied what Gurdjian (*15*) has termed the "preoptic region." In 4 other edematous animals the lesions were more caudally placed and were asymmetrical. In each instance, however, one needle puncture had penetrated the midline and bilaterally damaged the periventricular system. This suggests that the executant cell bodies or fibers in their caudal course occupy the region around the ventricle. The localization of the lesions in the control animals demonstrated that neither bilateral damage bordering the preoptic region nor unilateral damage to the preoptic region produced pulmonary edema.

Although the phenomenon described is by definition neurogenic hemorrhagic edema, whether it is neurogenic in the sense that the action is exerted on pulmonary vessels as opposed to the heart remains to be determined. That restricted lesions of the hypothalamus can cause such edema holds the promise that some of the nonspecific edemagenic procedures such as cerebral concussion may be given a unitary explanation.

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Physiological Availability of Dehydro-L-Ascorbic Acid and Palmitoyl-L-Ascorbic Acid[1]

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Previous studies of the biological activity of dehydro-L-ascorbic acid (DHAA) involved the use of solutions prepared by oxidation of natural or synthetic L-ascorbic acid (AA). The criteria for assessment of potency in guinea pig tests included cure of scurvy (*1–4*), weight gain (*3*), and increase in serum alkaline phosphatase (*5*). Johnson and Zilva (*6*) and Todhunter *et al.* (*7*) determined urinary excretions after oral dosage to humans. The relative activity of DHAA as compared to AA ranged from 80 to 100% in these tests. Recently, crystalline DHAA has been prepared by Pecherer (*8*) by a modification of the method of Kenyon and Munro (*9*) and made available to us; it was of interest to determine the activity of the pure compound in urinary excretion tests in humans similar to those described by Melnick *et al.* (*10*).

Seven male subjects participating in these tests consumed a self-selected diet, but followed a parallel diet on the 2 consecutive urine-collection days, particularly as regards foods high in AA. Since the diets were not standardized from week to week, considerable

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variations occur in some cases in the basal excretions, and each basal value applies only to its particular 2-day test. To insure saturation, all subjects ingested 300 mg of AA daily for 3 days, following which the supplement was discontinued or, preferably, reduced to 100 mg for the 2 days prior to collection as well as for the two 24-hr collection periods. The test dose of AA or DHAA was ingested in aqueous solution immediately after the noon meal on the second day. In 4 successive weeks each subject received 2 doses of both DHAA and AA. Since slight losses of DHAA were incurred in warming to 70° to dissolve the crystals in water, the potency of each dose solution of DHAA was determined by assay. Twenty-four-hour urine collections were started after the first voiding in the morning, and urines were kept in the refrigerator in amber bottles containing 5 ml of $5N$ H_2SO_4. Assays of both basal and test urines by the method of Rubin *et al.* (*11*) were begun within a few hours after collection was completed. The summary of the excretion data given in Table 1 shows pure DHAA to be completely available to humans.

TABLE 1

HUMAN AVAILABILITY OF CRYSTALLINE DEHYDRO-L-ASCORBIC ACID

Subject	24-Hour excretion of ascorbic acid in urine								
	L-Ascorbic acid dose (300 mg)				Dehydro-L-ascorbic acid dose (as indicated)				
	Basal (mg)	Test (mg)	Extra (mg)	Extra (%)	Basal (mg)	Test (mg)	Extra (mg)	Extra (%)	
EDR	24	96	72	24	26	165	139	50	(2)*
	48	199	151	50	106	197	91	31	(3)
MW	21	184	163	54	28	130	102	38	(1)
	67	227	160	53	39	241	202	70	(3)
JS	19	138	119	40	24	117	93	35	(1)
	40	177	137	46	42	155	113	41	(2)
FWJ	50	271	221	74	45	102	57	21	(1)
	61	110	49	16	64	177	113	41	(2)
JC†	318	810	492	55	281	732	451	56	(1)
	300	612	312	35	890	1395	505	61	(2)
WK	12	141	129	43	86	263	177	66	(1)
	38	170	132	44	20	180	160	58	(2)
ES	163	378	215	72	15	116	101	38	(1)
	157	358	201	67	98	354	256	93	(2)
		Average		48				50	
		± S.E.		± 4.5				± 5.0	

Availability of DHAA = 104 ± 14% (S.E. of quotient)

* Dose: (1) = 268 mg; (2) = 276 mg; (3) = 290 mg.
† Received three times the indicated dose because of extremely high basal excretions.

TABLE 2

URINARY EXCRETION OF DEHYDROASCORBIC ACID

Subject	Basal excretion	Excretion after oral dose	
		L-Ascorbic acid (300 mg)	Dehydro-L-ascorbic acid (268 mg)
	Mg dehydroascorbic acid per 24 hr		
EDR	1	25	15
MW	—	20	12
JS	6	19	12
FWJ	21	35	10
JC*	39	30	70
WK	—	52	23
ES	14	40	31
Average	16	32	25
± S.E.	± 6.7	± 4.1	± 8.1

* Received three times the indicated doses.

of either AA or DHAA is excreted as DHAA. Differences between the average excretions are not statistically significant.

One of the most widely used solvents for extraction of AA or DHAA is a 3% aqueous solution of metaphosphoric acid. For colorimetric assay with dichlorophenolindophenol, the extract is buffered to pH 3.9 with equal parts of glacial acetic acid and 50% sodium acetate (*11*). When this method is applied to crystalline DHAA, however, low recoveries are obtained consistently, regardless of whether H_2S reduction is continued for 2 hr or overnight. When the DHAA is dissolved in the pH 3.5 phthalate-hydrochloric acid buffer recommended by Kenyon and Munro (*9*), quantitative recoveries are obtained. Table 3 gives a summary of assay values found by 2-hr and overnight H_2S reduction of from 0.1 to 100 mg of a crude preparation in both types of buffer, and similar data for the 2-hr

TABLE 3

COMPARISON OF ASSAY PROCEDURES FOR CRYSTALLINE DEHYDRO-L-ASCORBIC ACID

Sample no.	Amount reduced (mg)	Colorimetric assay with dichlorophenol indophenol			
		Solution in 3% HPO_3 buffered to pH 3.9 with equal parts of glacial acetic acid and 50% sodium acetate		Solution in pH 3.5 phthalate-hydrochloric acid buffer	
		H_2S reduction			
		2 hr (%)	Overnight (%)	2 hr (%)	Overnight (%)
1 (Crude)	100	56	58	88	88
1 "	10	58	58	88	85
1 "	1	57	55	87	88
1 "	0.1	59	58	82	85
2 (Pure)	10	60	—	97	—

TABLE 4

HUMAN AVAILABILITY OF DEHYDRO-L-ASCORBIC ACID METHANOLATE AND OF PALMITOYL-L-ASCORBIC ACID

Subject	24-Hr excretion of ascorbic acid in urine											
	L-Ascorbic acid dose (300 mg)				Dehydroascorbic acid methanolate* dose (353 mg = 300 mg L-ascorbic acid)				Palmitoyl-L-ascorbic acid† dose (700 mg = 285 mg L-ascorbic acid)			
	Basal (mg)	Test (mg)	Extra (mg)	Extra (%)	Basal (mg)	Test (mg)	Extra (mg)	Extra (%)	Basal (mg)	Test (mg)	Extra (mg)	Extra (%)
EDR	63	193	130	43	86	233	147	49	70	236	166	58
LD	184	345	161	54	124	292	168	56	203	316	113	39
MW	90	304	214	71	90	275	185	62	116	195	79	28
RW	50	263	213	71	49	194	145	48	154	283	129	45
NC	171	356	185	62	218	326	108	36	119	300	181	64
EM	138	249	111	37	137	296	159	53	90	262	172	60
ES	240	385	145	48	163	290	127	42	60	249	189	66
JS	127	325	198	66	115	302	187	62	35	217	182	64
WM	204	300	96	32	239	386	147	49	94	273	179	63
			Average	54				51				54
			± S.E.	± 4.9				± 3.2				± 4.5

Availability of DHAA · MeOH = 94 ± 10% (S.E. of quotient)
Availability of PAA = 100 ± 12% (S.E. of quotient)

* Assayed 85% as DHAA = 100% as DHAA · MeOH.

† Assay 97.5% on a moisture-free basis.

reduction of 10 mg of a pure preparation. It is evident that the HPO_3-acetate method is not applicable in the assay of crystalline DHAA. No evidence is available to show that similar losses of DHAA are encountered in the extraction of natural products with 3% HPO_3. It is very likely that natural buffers afford protection to AA and DHAA during HPO_3 extraction. Attempts to compare the two extractants have failed because the phthalate-hydrochloric acid buffer does not provide sufficient protection to reduced ascorbic acid during such extractions and is generally unsatisfactory since it is not a strong enough buffer in many cases to change the color of the indophenol dye from blue to red.

A crystalline complex of DHAA and methanol (1 to 1) has also been described by Pecherer (*8*). This compound (DHAA · MeOH) is of interest because its solubility in water is much greater than that of DHAA. Solution occurs rapidly at room temperature with no loss of potency in either water or 3% HPO_3. In acute oral toxicity tests in mice, DHAA · MeOH was found to be no more toxic than AA or DHAA, the LD_{50} being in excess of 5 g/kg. In a short-term chronic toxicity test, mice tolerated 7 daily doses of 1 and 2 g/kg orally with no apparent toxic effect. The results of a human availability test by the above urinary excretion technique are summarized in Table 4 and indicate complete availability of DHAA · MeOH within the accepted limits of the method.

The availability of palmitoyl-L-ascorbic acid (PAA) to humans has also been determined by the urinary excretion technique, because of the limited scope of previously reported data on its biological activity. Ambrose and De Eds (*12*) found normal serum phosphatase levels after administration of 0.5 mg/day of AA or an equivalent amount of PAA to scorbutic guinea pigs for 5 days, the water-insoluble PAA being fed as a solution in propylene glycol. These tests did not provide rigid proof of the biological equivalence of AA and PAA since it was not determined whether the doses given were suboptimal. Goswami *et al.* (*13*) fed the same equivalent levels of AA and PAA to scorbutic guinea pigs for 2 weeks and found average weight gains of 27 and 28 g, respectively, whereas the negative controls died of scurvy. Although this assay indicated equivalent antiscorbutic activity of the two compounds, the number of animals was limited to 2 for AA and 3 for PAA. It is not stated whether the latter was given as a solid or in solution. In the present human availability test, the PAA was given in the solid form as a suspension in water. Comparative excretions of AA are summarized in Table 4 and indicate 100% availability of the palmitate ester. Since the tests of PAA and DHAA · MeOH were run several months after the DHAA tests, it was considered desirable to repeat the standard dose of AA. Thus, the 3 tests in Table 4 were carried out in successive weeks. It may be noted that the average excretion after AA dosage is in close agreement with the average given in Table 1 for previous tests.

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A Satisfactory Apparatus for Study of Analgesia in Mice

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The white mouse is a suitable animal for the study of the threshold to induced pain because if dropped upon a heated surface it will sharply withdraw its paws when the sensation of heat is succeeded by pain, the time lapse before this withdrawal being quite constant in individual animals. The only difficulty in our experience has been to obtain a constant and uniformly heated surface upon which to drop the mouse, a handicap now overcome through the development of the herein described apparatus, which is a modification of an arrangement discussed with one of us (H. B.) by N. B. Eddy, of the National Institutes of Health, who based his method upon that described by Woolfe and MacDonald (*1*).

The apparatus, shown in Fig. 1, consists of a copper teakettle, the large opening of which is sealed with a copper plate and the spout of which is connected with a long glass condenser through a tightly fitted cork and a copper cylinder soldered into the spout. The kettle is heated by a 600-w element operated through an adjustable bimetallic relay system and is about two thirds filled with a mixture of equal parts of ethyl formate and acetone. The mixed fumes from the ethyl formate (bp, 54°–55.5° C) and acetone (bp, 55.5°–55.8° C) are returned from the condenser to the kettle, and the temperature of the soldered-in copper plate in the top of the latter is maintained uniformly at 54°–55° C, which is just the pain temperature threshold of the mouse.

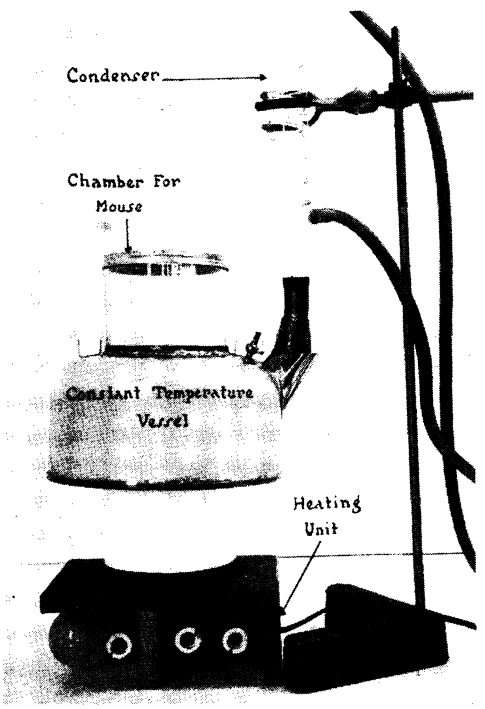

FIG. 1. Apparatus for study of analgesic threshold in mice.

To keep the mouse in contact with the hot plate during "pain-point" determination, a glass cylinder with a cover is fixed on top of the plate. The simultaneous lifting and licking of both forefeet have served us as the most reliable "pain-point," all mice invariably showing this sign within 15 sec after being put on the plate. In our experience any agent deferring the "pain-point" beyond 15 sec may be considered an analgesic.

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Stress and Ketone Body Metabolism[1]

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A clue to the role of fat in the metabolic adjustments of man to systemic stress has been provided by a review of data on ketonuria observed during Army ration trials and nutrition surveys. As a part of the nutritional and metabolic assessment of the troops taking part in the trials and surveys conducted in the period 1944–50, specimens of urine were collected for routine testing by one or another modification of Rothera's method (*1*) for detecting the presence of nitroprusside positive substances. The positive reactions, which were identical with those originally described by Rothera (*1*), were qualitatively graded: trace and 1 to 4+. In general the results have been reported as percentages of the tested samples that were positive at any given time (to be called here "per cent ketonuria").

The specimens of urine were collected under a variety of conditions. In the nutrition surveys, the specimens represented random samples. Occasionally similar samples were taken on ration trials. Usually,

[1] The opinions expressed in this paper are those of the authors and do not necessarily represent the official views of any governmental agency.

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