serum. In particular, the proportion of phosphatidyl ethanolamine in the two ruminants was apparently very low. This finding is consistent with the general impression that the hog is physiologically closer to man than are several other domestic animals, and may reflect the omniverous, non-ruminant mode of nutrition of the two species.

These results suggest that the phospholipids of hog serum may provide an efficient readily available and physiologically acceptable emulsifying agent for use in preparation of intravenous fat emulsions. An emulsion made with hog serum phospholipids as the only emulsifying agent is under investigation.

Summary. Thin-layer chromatography has been used to gain a roughly quantitative impression of the phospholipid distribution within the human chylomicron and of the phospholipid mixture present in various animal materials. The phospholipids of chylomicrons obtained following a fat meal were similar in distribution to those of total human plasma, and comprised phosphatidyl choline, sphingomyelins, phosphatidyl ethanolamine and lysophosphatidyl choline. Hen egg-yolk phospholipids showed a higher content of phosphatidyl ethanolamine but lower amounts of sphingomyelins. The sera of the sheep, steer and hog were examined, and of these the phospholipids of the last were closest in composition to those of the chylomicron. It is suggested that hog serum phospholipids may provide a physiologically acceptable emulsifying system for use in intravenous fat emulsions.

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Occurrence of (-)-Sympatol in Oranges.* (28635)

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p-Hydroxymandelic acid is a constant constituent of urine(1) and the amount excreted daily does not appear to fluctuate markedly. Octopamine (*p*-hydroxy-*α*-(aminomethyl) benzyl alcohol) was suspected of being an endogenous precursor for this acid, and was identified in tissues of animals which had been treated with monoamine oxidase inhibitors and in the urine of man and animals(2). In a more extensive survey of the phenolic amines in human urine *p*-sympatol, the N-methyl derivative of octopamine, was identified(3). It occurs only in the conjugated amine fraction and the amount present varies widely from day to day. For this reason it was suggested that it probably has a dietary source(3). However, Pisano *et al.*(4) suggested that *p*-sympatol might be of physiological significance, and Kraupp *et al.*(5) reported its occurrence in increased amounts in the urine of a patient with hypertension. Because of their reports and also because the presence in urine of increased amounts of *p*-sympatol or its metabolite, *p*-hydroxymandelic acid, might interfere with some of the tests used for diagnosis of pheochromocytoma the existence of a common dietary source of *p*-sympatol is reported here.

Human subjects maintained on rigidly defined diets(6) were observed to excrete markedly increased amounts of *p*-hydroxymandelic acid after they ingested oranges or orange juice. Further examination of their urine showed the presence of a large amount of a phenolic amine which had the chromatographic properties of *p*-sympatol. The results of an experiment made to extend this observation were as follows: A healthy adult male took a diet free of precursors of phenolic

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acids and phenolic amines. On the third day, during an 8-hour period beginning at 9 A.M. he excreted 2.0 mg of *p*-hydroxymandelic acid and no *p*-sympatol. The next morning he ate 1 kg of oranges (California Navel) from which the peel had been removed. During the next 8 hours he excreted 9.5 mg of *p*-hydroxymandelic acid and 4.0 mg of *p*-sympatol. All the *p*-sympatol was in the conjugated amine fraction. These results indicated that a minimum of 11.5 mg of *p*-sympatol must have been present in the 1 kg of oranges ingested.

Next, 1 kg of the same batch of oranges was blended with 1 l of cold 0.2 N HCl, the mixture was centrifuged, the supernatant was separated and saved, and the residual pulp was treated again in the blendor with an equal volume of 0.2 N HCl. The total volume of the combined extracts was 2300 ml. A 5 ml portion of this extract was examined for its content of phenolic amines with the same procedure as used for urine(3). The chromatograms showed the presence of a large amount of *p*-sympatol and very much smaller amounts of some other phenolic amines which were not examined further, All of the *p*-sympatol in the orange extract was in the free form. A quantitative estimate showed that the extract contained 45 mg of *p*-sympatol, which corresponds to a concentration of 4.5 mg per 100 g in the orange.

Difficulties were encountered in handling the acidic extract because of gel formation so it was not possible to isolate all of the *p*-sympatol in the extract. The extract was neutralized to pH 5, diluted, and clarified by centrifugation, and the amines and amino acids were separated and concentrated by adsorption onto a column of Amberlite CG-120 (H^+) (100-200 mesh) followed by elution with 3 N NH_4OH. The eluate was concentrated to dryness *in vacuo*, the syrupy residue was taken into 100 ml of water, adjusted to pH 6 and the amines were separated by adsorption onto a 1.5 × 20 cm column of Dowex 50-X2 (H^+). The column was washed with 0.1 N sodium acetate solution to elute amino acids, which were detected by testing portions of the effluent with ninhydrin. The wash was continued until a negative test was obtained. The resin was washed with water and the amines were then eluted with 1 N NH_4OH in 65% ethanol. Arginine was the only amino acid which remained in the amine fraction. This eluate was concentrated to dryness, the residue was dissolved in 0.1 M phosphate buffer, pH 8.6, and applied to a 2 × 35 cm column of Amberlite IRC-50 (100-200 mesh), which had been conditioned with the same buffer. Amines were eluted with the same buffer, using a flow rate of approximately 1 ml/min.; their emergence was detected by measurement of the absorption of the fractions at 272 mμ. Authentic and natural *p*-sympatol both emerged between 425 and 600 ml, with the peak at 450 ml. Material from the center 3 ml was used to compare the ultraviolet, fluorescence, and activation spectra of authentic and natural *p*-sympatol in acid and in base as described earlier(3). They were identical. The eluate between 425 and 575 ml was pooled and desalted by adsorption onto Dowex 50-X2, and the resulting *p*-sympatol was dissolved in 3 ml of water. The amount present was determined spectrophotometrically to be 11.2 mg. The rotation of the material was then determined; α_D^{29} −20.2° (c, .37, H_2O). This showed the natural *p*-sympatol to be levorotatory and probably of the *D* configuration, as judged by comparison with the properties of other mandelamines of known configuration. Pure *p*-sympatol has a specific rotation of 61.5° in water(7), but it is known to undergo racemization readily in acid solution(8). Because of the difficulties encountered in handling the original acid extract of oranges, the solution stood for several weeks in the cold room, so the 30% racemization which occurred is not unexpected.

In addition to the properties reported above, authentic and natural *p*-sympatol had the same chromatographic properties as described earlier(3).

Several varieties of oranges, commercial orange juice, and frozen concentrates were examined. All contained free *p*-sympatol, and also very small amounts of a substance which has the chromatographic properties of octopamine. Lemons and several varieties of grapefruit were examined but were found to contain no *p*-sympatol.

Orange extract was also examined for the

presence of *p*-hydroxymandelic acid, using the procedure described for detecting phenolic acids in urine(1). None could be detected.

For a more accurate estimation of the *p*-sympatol content of an orange, a Florida orange (188 g) was separated into peel (30 g) and pulp (158 g). Separately, they were blended thoroughly with an excess of 0.2 N HCl, the suspension of peel was diluted to exactly 500 ml and the pulp to one l and filtered. 0.1 ml of the peel extract and 0.5 ml of the pulp extract were diluted to 5 ml with water, and the *p*-sympatol content was determined by separating amines(3) and comparing the amount in the extract with known amounts of authentic amine on one-dimensional paper chromatograms using isopropyl alcohol-conc. NH_4OH-water (8:1:1) as the solvent system. The peel was found to contain 10.0 mg and the pulp 6.0 mg of *p*-sympatol, all in the free form. Peel and pulp both contained in the free amine fraction a very small amount of a material which had the properties of octopamine.

The amino acids present in the concentrate eluted from Amberlite CG-120 resin were examined with paper chromatography and with an amino acid analyzer. Oranges proved to contain much γ-aminobutyric acid, as had been reported previously(9,10). Sufficient was present that some was excreted by the person who ate a kilogram of orange pulp. For quantitative analysis, 10 ml of the extract of pulp, and 100 ml of the peel extract, respectively, were passed through a 2 × 10 cm column of Amberlite CG-120 (H^+) (100-200 mesh), the resin was washed with 100 ml of water, and amino acids were eluted with 50 ml of 3 N NH_4OH. The ammoniacal eluates were concentrated to dryness *in vacuo* at room temperature and dissolved in exactly 10.0 ml of pH 2.2 citrate buffer, 0.2 N in Na^+. A 2 ml aliquot of each was used for amino acid analysis with a Beckman/Spinco Model 120 amino acid analyzer, using the conditions of Spackman *et al.*(11). The results of the analysis are given in Table I. It should be noted that this procedure does not distinguish between glutamine and asparagine, which are eluted together, and are reported as glutamine in the Table. However, the acid extraction and the treatment with base for elution from the resin probably resulted in hydrolysis of a substantial portion of the glutamine in the orange. The pulp of the orange may be seen to contain considerably more free proline, arginine, and γ-aminobutyric acid than any of the other amino acids.

TABLE I. Free Amino Acid Content of an Orange.

Amino acid	Pulp (mg)	Peel (mg)
Aspartic acid	39.4	4.92
Threonine	2.98	.30
Serine	16.3	.19
Glutamine + asparagine	31.1	.44
Proline	146.2	30.8
Glutamic acid	19.1	3.09
Glycine	2.48	.20
Alanine	12.11	2.40
Valine	4.56	.40
Methionine	.36	
Isoleucine	1.34	
Leucine	1.39	
Tyrosine	2.16	.18
Phenylalanine	4.72	.23
γ-Aminobutyric acid	72.2	3.24
Ornithine	.66	.09
Ethanolamine	.88	.37
Lysine	5.92	.25
Histidine	2.72	.26
Arginine	114.1	.95

Orange juice has been used frequently as the carrying medium for administration of compounds to subjects for various metabolic studies. In addition, it is a common item on hospital menus. The presence of *p*-sympatol and γ-aminobutyric acid should be taken into account in any studies involving these or related substances.

Summary. Oranges contain a considerable amount of free *p*-sympatol. The ingestion of orange juice results in excretion of conjugated *p*-sympatol and *p*-hydroxymandelic acid.

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Spectrophotometric Studies of Peritoneal Fluid in Strangulation Intestinal Obstruction.* (28636)

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Nemir and colleagues(1) demonstrated a hemin pigment in the loop fluid, peritoneal fluid, and blood of dogs just prior to death in experimental intestinal strangulation obstruction. There was no evidence that the spectrophotometrically observed pigment was the cause of death. It was suggested that the toxic agent appeared in the peritoneal fluid at approximately the same time as the changed pigment, and that the course of the toxic agent could be followed through that of the pigment. Subsequently, the pigment was observed in association with gangrenous intestine in but one of 18 clinical cases(2). Evaluation of the importance of the abnormal pigment in the pathogenesis of strangulation obstruction is difficult in view of the negative findings in clinical cases(3,4). A similar abnormal hemin pigment was observed in the plasma of dogs prior to death in irreversible hemorrhagic shock(3). Katz and colleagues (6), who were unable to demonstrate the abnormal pigment in experimental strangulation obstruction, concluded that any possible lethal action of strangulation fluid did not depend on the altered hemoglobin.

Both oxyhemoglobin and the abnormal pigment are characterized by a biphasic spectral absorption curve over the range 525 to 600 mμ. When a strong reducing agent (viz., sodium dithionite) is added, oxyhemoglobin is deoxygenated and yields a monophasic curve characteristic of reduced hemoglobin. With addition of dithionite to the abnormal pigment, a biphasic curve persists indicating an irreversible change in the hemoglobin. During a survey of strangulation loop fluids and peritoneal fluids, we observed a pigment, apparently similar to that initially reported, in the terminal peritoneal fluid of one dog(4). The presence of this pigment was not related to either bacterial flora or lethality of the peritoneal fluid. This pigment is of biochemical interest, and spectrophotometric studies demonstrating a relationship between the abnormal pigment and hemochromogen are described here.

Materials and Methods. Spectral absorption curves were obtained by reading optical density of appropriately diluted specimens in a Beckman DU spectrophotometer(4). Alkaline oxyhemoglobin was deoxygenated with sodium dithionite (sodium hydrosulfite, purified, J. T. Baker Chemical Co., Phillipsburg, N.J.). Total hemoglobin was determined by the cyanmethemoglobin method. Alkalinity was measured with a Beckman H-2 pH meter. The peritoneal fluid containing the abnormal pigment was aspirated from Dog #5430 which died 30 hours after a closed loop strangulation obstruction was created in a 30 cm segment of ileum(4). Dog #5432 was subjected to the same surgical procedure on the same morning and died 41 hours later.

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