Table 1. Characteristics of mercury and gallium dropping from the same capillary; $r^4/l = 1.13 \times 10^{-12}$ cm³ for the capillary.

Characteristics	Mercury		Gallium	
	Air	0.1*N* KCl	Air	0.1*N* KCl
P (cm Hg)	14	14	24	24
W (mg)	32.3	4.84	72	11
t (sec)	4.77	0.71	26	3.8
m (mg sec^{-1})	6.76	6.82	2.8	2.8
$m^{2/3}t^{1/6}$	4.53	3.35	3.1	2.3
P/m	2.06	2.06	19	19
v ($\times 10^{-3}$ ml)	2.38	0.36	12	2

law. On substituting the appropriate numerical values for the coefficient of surface tension, 600 dynes cm^{-1}, and the coefficient of viscosity, 2.09 centipoises (*3*), the equation becomes

$$m = \frac{6.84 \times 10^8 r^4}{l}\left(h' - \frac{7.9}{m^{1/3}t^{1/3}}\right),$$

where h' is expressed in terms of centimeters of gallium. In general, the drop time seemed to vary much more with the applied voltage than in the case of mercury, although the electrocapillary curves of both metals have about the same shape (*4*, *5*). Also the size of capillary bore capable of producing reasonable rates of flow was found to be rather critical.

The only current-voltage curve that could be fairly well reproduced was obtained with an air-saturated 0.1*N* KCl solution (Fig. 2). It shows the following features: the dropping gallium electrode has a potential of −1.0 v (versus saturated calomel electrode) as indicated by the value of applied emf at zero current. Thus it is anodically polarized at lower applied voltage. The portion of the curve corresponding to that region is quite similar to that for mercury with the difference that the current intensity is much greater, as explained in the preceding paragraph.

On the cathodic polarization side, a fairly well defined wave occurs with a half-wave potential of −1.26 v.; the current at that point was of the order of 30 μamp. Since that wave disappeared entirely in an oxygen-free solution, it corresponds probably to the reduction of the gallic ions produced by reaction of the metal with dissolved oxygen. This conclusion is borne out by the fact that the cathodic half-wave potential is the same as that of the gallic ion (*6*, *7*). Beyond −1.6 v, the current increased sharply owing to hydrogen evolution. Because of the numerous difficulties encountered, no attempt was made to study the reduction of other metal ions at the dropping gallium electrode.

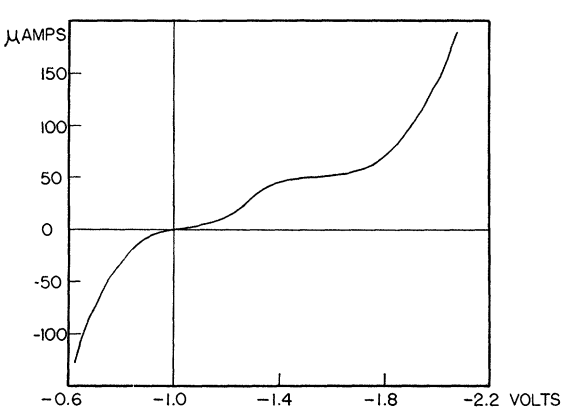

Fig. 2. Current-voltage curve for the dropping gallium electrode in air-saturated 0.1*N* KCl solution.

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Identification of the So-called "Lard Factor" as Vitamin A*

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Discovery of a new vitamin was announced by Kaunitz, Slanetz, and Johnson in 1950 (*1*). It could be concentrated by molecular distillation of freshly rendered lard and was designated the "lard factor." The scope of this claim was subsequently modified, and reports from the same laboratory (*2–5*) showed that the disease described was simply classical vitamin-A deficiency. The new factor was then considered to be a substance possessing biological activity of vitamin A but differing chemically from known forms of vitamin A. The content of vitamin A was claimed to be less than 10^{-7} g per gram of distillate (0.33 IU/g) as the result of analyses by the usual chemical and spectrophotometric methods.

Lowe, Morton, and Harrison (*6*) and Lowe and Morton (*7*) bioassayed a similar lard distillate and found approximately 10 IU of vitamin-A activity per gram. They reported that "Neither preformed vitamin A nor carotenoid provitamin A could be detected by spectrophotometric or colorimetric tests on lard or lard unsaponifiable matter before or after chromatography, or on lard distillate," and concluded that the existence of the "lard factor" was confirmed.

Conversely, Engel (*8*) reported no indication of the presence of a "lard factor" in fresh lard that he fed to vitamin-A-depleted rats at a 10-percent level in the diet. Recently Herb, Riemenschneider, Kaunitz, and Slanetz (*9*) reinvestigated two lard distillates and found indications of the presence of vitamin A. These

small amounts of vitamin A were estimated by spectrophotometric analysis of the chromatographed unsaponifiable fraction. Consequently, these investigators withdrew their previous claims and concluded that "typical vitamin A is present in lard and probably accounts for most of its biological vitamin A activity." The existence of the lard factor was considered unlikely but was "not completely excluded."

The results of our investigations have established that the so-called lard factor in molecular distillates of lard is in fact vitamin A. We acknowledge with thanks the cooperation and assistance of the members of the Vitamin Development and Manufacturing Control Laboratories of DPI with this investigation.

We have analyzed various lard distillates colorimetrically following careful saponification and found a range of apparent vitamin-A content from 5.5 to 28 units/g, corresponding to 0.5 to 2.5 units/g in the original lard sample, as is shown in Table 1.

Table 1. Vitamin-A analyses of lard distillate.*

Sample No.	Distillate fraction of original input lard (%)	Unsaponifiable matter in distillate (%)	Vitamin A in distillate (units/g)	Calculated vitamin A in original lard (units/g)
D-4-80	10.1	1.67	6	0.6
D-4-99	8.6	2.64	7.8	0.9
D-Q-614	9.0	4.36	5.5	0.6
25104	13.0	1.9	7	0.5
25104	13.0	1.0	6.8	0.5
65009	11.4	2.2	17	1.5
65017-1	11.0	1.82	28	2.5
65017-2	11.0	1.84	22	2.0

* Freshly rendered lard was molecularly distilled at 215°C and 10^{-3} mm Hg pressure. The first fraction, representing approximately 10 percent of the original lard, was removed, saponified, and assayed for vitamin A by the Carr-Price reaction. It is impossible to determine accurately the small amounts of vitamin A present by the USP XIV procedure involving the Morton-Stubbs correction for extraneous absorption. The stills were carefully cleaned to eliminate the possibility of chance contamination of these distillates with vitamin A.

Additional evidence of the identity of vitamin A in lard distillates was obtained, as is shown in Table 2. A lard distillate and a sample of the unsaponifiable matter from the same lard distillate were bioassayed by the standard USP method. The two preparations were analyzed by the increment blue color test as described in the USP XIV. This technique corrects for any inhibition of the Carr-Price blue color by the test material and is generally considered to be a good identity test for vitamin A. It appears from the results of this test that all the bioactivity can readily be accounted for by chemically measured vitamin A present.

Positive identification of vitamin A in the two distillates was made by applying the anhydrous HCl dehydration procedure of Shantz, Cawley, and Embree

Table 2. Vitamin-A content of lard distillates determined by bioassay and physicochemical methods.

Type of analysis	Sample: Lard distillate (25104)	Sample: Unsaponifiable fraction (RL-11-20-51) of lard distillate (25104) in cottonseed oil*
Biopotency ± S.E.† (units/g)	5.01 ± 1.18	62.5 ± 9.9
Increment blue color value‡ (units/g)	6.18	135
Dehydration values§ (units/g)	4.75	104

* The unsaponifiable fraction of lard distillate in oil solution was obtained as follows: 700 g of distillate was saponified for 2 hr in 105 ml of ethanol with 210 g of 85 percent KOH in 350 ml water. The mixture was diluted with 5 lit water and extracted 5 times with a total of 8½ lit of ethyl ether. The ether extracts were concentrated to 250 ml, a 50-ml aliquot removed for analysis, and 20.0 g of refined cottonseed (Wesson) oil added to the remaining 200 ml. The ether was then removed under vacuum, leaving a solution of unsaponifiable matter in cottonseed oil.

† Rat growth bioassays were routine tests in which two levels of test substance were compared with two similar levels of the USP Vitamin A Reference Standard. A modified USP XIII bioassay procedure for vitamin A was employed, as suggested by Bliss (*12*).

‡ The potency by increment blue color was determined as outlined in the USP XIV.

§ A portion of the unsaponifiable fraction was treated with alcoholic HCl, and the resulting anhydrovitamin A was determined as described by Shantz *et al.* (*10*).

(*10*). This dehydration reaction has been used successfully to prove the presence of vitamin A in human blood extracts (*11*). It consists essentially of subtract-

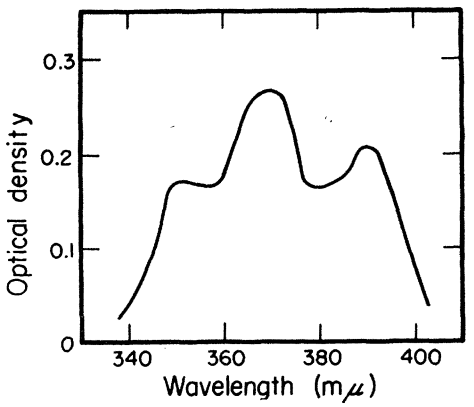

Fig. 1. Spectral absorption curve for anhydrovitamin A by dehydration of the unsaponifiable matter from lard distillate. Maximums were observed at 351, 370, and 391 mμ, compared with 351, 371, and 392 mμ for pure anhydrovitamin A. Absorption ratios for pure anhydrovitamin A are 351 mμ/371 mμ = 2500/3650 = 0.68 and 392 mμ/371 mμ = 3180/3650 = 0.87. Corresponding ratios for the anhydrovitamin A from lard distillate of 0.64 and 0.77, respectively, are in excellent agreement.

ing the U.V. absorption curve of the extract suspected of containing vitamin A from the U.V. absorption curve of the extract after the dehydration reaction. If the "difference curve" is the characteristic triple-peak absorption curve of anhydrovitamin A, the presence of vitamin A in the original extract is proved. The absorption curve of the anhydrovitamin A prepared from the unsaponifiable fraction of lard distillate is shown in Fig. 1. This establishes the presence of vitamin A, per se, in the preparation. The results of these determinations give no indication of more vitamin-A bioactivity than can be determined as vitamin A by conventional chemical analyses. The failure of some investigators to find vitamin A in lard concentrates may have been due to loss during saponification of the small amounts of vitamin A present.

Thus, the postulated "lard factor" of Kaunitz and coworkers has been identified as vitamin A. Vitamin A, per se, has been found in amounts sufficient to account for the vitamin-A bioactivity of lard distillates.

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* Communication No. 201 : No. XI of a series entitled "Biochemical Studies on Vitamin A.

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Graphitization of Diamond

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There have been numerous investigations of the graphitization of diamond under a variety of conditions (*1–19*). From the literature it appears that the diamond-graphite transition rate (in vacuum or inert atmosphere) is zero or immeasurably slow below 1000°C, measurable from 1000° to 1500°C, but at any given temperature has not been reproducibly measured. The temperature at which the transition rate is first measurable has not been established.

An attempt has been made in this laboratory to extend the data on the transition. Each diamond was heated in an atmosphere of dry helium (99.95 percent pure with 0.05 percent H_2). The sample was placed in a recrystallized alumina crucible fitted with alumina lid which was encased in a graphite crucible and lids. This crucible assembly was suspended in a gas-tight mullite tube through which the dry helium was passed. A protected Pt-Pt 10 percent Rh thermocouple adjacent to the crucible was used to follow the temperature.

Clear, industrial-grade diamond macles that were free from obvious inclusions were selected. These weighed 10 to 20 mg each, were triangular shaped, were approximately 1 mm thick, and had 2- to 3-mm equilateral sides. Individual experiments, summarized in Table 1, were as follows:

Table 1. Graphitization of diamond.

Wt. of diamond (mg)	Temp. (°C)	Time (hr)	Percentage loss ± 0.2%	Microscopic examination after heating
16.82	1000	16	0.0	No graphitization
16.82	1100	17	.0	
11.88	1200	24	.0	
11.88	1200	50	.0	
18.70	1200	24	.0	
18.57	1300	50	.2	Superficial graphitization
17.14	1400	50	1.1	Black coating
17.18	1400	6	0.0	Superficial blackening
17.38	1400	24	.6	Black coating
15.24	1400	49	1.2	
11.76	1400	49	2.1	
19.76	1400	48	1.3	
9.71	1400	48	2.1	

1) A diamond was cleaned with acetone, dried, and weighed to ± 0.01 mg.

2) It was placed in the alumina crucible encased in the graphite crucible. The crucibles were covered with lids of alumina and graphite, respectively, and the assembly was placed in the furnace.

3) The helium flow was started and the furnace was equilibrated at the desired temperature.

4) At end of the desired heating time, the furnace was shut off and cooled to room temperature with helium flow continued.

5) The diamond was removed, weighed to ± 0.01 mg, and viewed under a microscope to determine the nature and extent of any change. In some cases x-ray diffraction studies of the surface were made.

6) The diamond was treated with hot perchloric acid and ammonium vanadate to remove any graphite formed.

7) The diamond was cleaned with acetone and reweighed to ± 0.01 mg. The loss in weight was taken as the percentage of graphitization.

A blank on the original material showed no loss in weight upon treatment with hot perchloric acid and ammonium vanadate. The heating time is taken from the time the furnace reached the specified temperature until the heat was turned off. Approximately ½ to 1 hr was needed to reach the specified temperature and approximately ½ hr to cool below 1000°C when the heating current was cut off. The temperatures were maintained within 15°C of those shown in Table 1.

Diamond powders (40–50, 120–140, 230–270, 500, and 4000 mesh) were also heated but with no clear-cut