

effort of producing amboceptor, an effort which the body cells of a patient sick with typhoid fever may be unable to accomplish.

8. There is not the same liability of producing what is called a "negative phase"—the temporary condition sometimes following the injection of an ordinary bacterin owing to the using up of the normal opsonin in the patient's blood, the same being required to prepare the injected bacterin for phagocytosis.

PASSIVE IMMUNITY-SERUM THERAPY.

So called because the body cells of the individual undergoing immunization have no part in producing it. Active immunity is produced in some other animal, usually the horse, and the individual to be passively immunized acquires immunity by receiving injections of immune serum taken from the immunized animal. The use of diphtheria antitoxin to prevent diphtheria (prophylactic immunization), and as a curative agent in its treatment (curative immunization) illustrates the main purposes for which passive immunization is employed.

Although sufficient evidence has not yet accumulated to permit positive statements, it is believed that more than one antibody is usually present in each immune serum, and that the immunity acquired by their employment is due to several antibodies, which, acting together, tend to overcome infection.

As stated by Kolmer⁵, "From the practical standpoint, therefore, immune serums may be used to produce two main types of passive immunization, namely:

"1. *Antitoxic immunization*, due to antitoxins opposing the true extra-cellular toxins, as in diphtheria and tetanus (antitoxic immunity).

"2. *Antibacterial immunization*, due mainly to bacteriolysins and bacteriotropins (antibacterial immunity)." Serums containing these antibodies are useful in the treatment of infections due to the meningococcus, pneumococcus, streptococcus, gonococcus, etc.

I am forced to end this paper abruptly to permit its publication in the JOURNAL. It is my intent to continue the subject in subsequent papers.

STABILITY OF PREPARATIONS CONTAINING YELLOW PHOSPHORUS.*

H. ENGELHARDT AND O. E. WINTERS.

In our previous paper entitled the "Estimation of Yellow Phosphorus"¹ read at the Detroit meeting we announced our intention to substitute other copper salts for copper nitrate, which we used in the process suggested.

The tabulated results of this work show quite plainly that no advantage is gained by the use of other salts of copper over the nitrate.

With the exception of those made with copper chloride, the results obtained when employing other copper salts were fairly uniform. Copper chloride is the least appropriate for this purpose, due to the apparent decomposition of the

⁵A Practical Text-book of Infection, Immunity and Specific Therapy, by John Kolmer, M.D., Dr. P.H., Phila. W. B. Saunders Co., 1915.

* Read in Scientific Section A. Ph. A., San Francisco meeting.

¹ Journal A. Ph. A., Apr. 1915, p. 451.

copper compounds, especially copper phosphide, which takes place. Again we have found that the direct copper nitrate method as outlined in our previous paper gives trustworthy results. We also found that it is advisable and important to collect the ammonium-magnesium phosphate in a Gooch crucible, because less wash-liquid is required to remove the excess of magnesia mixture and the danger of dissolving the ammonium-magnesium phosphate is consequently practically eliminated.

APPLICATION OF VARIOUS COPPER SALTS.

2.837 gms. of dried phosphorus was dissolved in 250 cc. of air-free chloroform and 10 cc. of this solution, equal to 0.1135 gms. of phosphorus, was treated with copper nitrate solution directly. The following results were obtained:

.392 gm. $Mg_2P_2O_7$	=96.45 per cent of the theoretical amount.
.393 " " "	=96.7 " " " " "
.400 " " "	=99.51 " " " " "

The same quantity of the chloroformic phosphorus solution was then treated with copper nitrate solution, the copper phosphide was filtered as outlined in our previous paper and both the nonoxidized phosphorus and the oxidation products were estimated with the following results.

<i>Magnesium pyrophosphate from phosphide</i>	<i>Magnesium pyrophosphate from filtrate</i>
.268 gm. = 65.94 per cent phosphorus	.117 gm. = 28.78 per cent phosphorus
.255 gm. = 62.73 per cent phosphorus	.136 gm. = 33.46 per cent phosphorus
or combined 94.72 per cent	
96.19 per cent	

The 10 per cent copper nitrate solution was then replaced by a 5 per cent copper acetate solution and the following results obtained which agree fairly well with those obtained by the copper nitrate method.

<i>Magnesium pyrophosphate from phosphide</i>	Or combined
.258 gm. = 63.48 per cent phosphorus	93.5 per cent
.257 gm. = 63.23 per cent phosphorus	98.15 per cent
.252 gm. = 62.1 per cent phosphorus	97.72 per cent
<i>Magnesium pyrophosphate from filtrate</i>	
.122 gm. = 30.02 per cent phosphorus	
.142 gm. = 34.92 per cent phosphorus	
.145 gm. = 35.62 per cent phosphorus	

When substituting the 10 percent copper nitrate solution by a 10 percent copper sulphate solution the results obtained showed that a greater oxidation of the copper phosphide takes place during the process than when using the nitrate or acetate.

<i>Magnesium pyrophosphate from phosphide</i>	
.199 gm. = 49.0 per cent phosphorus	
.202 gm. = 49.7 per cent phosphorus	
.215 gm. = 52.9 per cent phosphorus	
<i>Magnesium pyrophosphate from filtrate</i>	Or combined
.192 gm. = 47.4 per cent phosphorus	96.4 per cent phosphorus
.182 gm. = 44.8 per cent phosphorus	94.5 per cent phosphorus
.180 gm. = 44.4 per cent phosphorus	97.3 per cent phosphorus

The oxidation was still more marked when using a 10 percent copper chloride solution.

Magnesium pyrophosphate from phosphide

.189 gm.	= 46.5	per cent phosphorus
.139 gm.	= 34.2	per cent phosphorus
.054 gm.	= 13.3	per cent phosphorus

Magnesium pyrophosphate from filtrate

.187 gm.	= 45.9	per cent phosphorus
.205 gm.	= 50.4	per cent phosphorus
.359 gm.	= 88.0	per cent phosphorus

Or combined	92.4	per cent phosphorus
	84.6	per cent phosphorus
	101.3	per cent phosphorus

These results are absolutely worthless. As yet we have not been able to find out the cause of these discrepancies. We have made several sets of assays under exactly the same conditions but the results obtained were as varying as those reported. When using copper salts other than the chloride the precipitate of the copper phosphide has a black color, while the chloride produces a precipitate which at first is black but which soon changes to brown, the color of copper phosphide generally described in the text-books on chemistry.

STABILITY OF SPIRIT OF PHOSPHORUS.

Since the results obtained with copper sulphate and copper chloride were unsatisfactory, we used only the nitrate and acetate in the estimation of the spirit. The estimation was carried out as given in our previous paper, using 10 cc. of the spirit for the estimation.

The following results were obtained:

Copper nitrate	0.0505 gm.	$Mg_2P_2O_7=0.141$	per cent phosphorus.
"	"	"	"
"	0.050	"	"
"	0.0508	"	"
Copper acetate	0.049	"	"
"	0.049	"	"

In another series of experiments the oxidation products were not removed by shaking out with water, 10 cc. of the spirit were simply mixed with 25 cc. of chloroform, 40 cc. of water and 20 cc. of copper nitrate and copper acetate solution respectively. We thus obtained the following results:

Copper nitrate	0.065 gm.	$Mg_2P_2O_7=0.183$	per cent phosphorus.
"	"	"	"
"	0.068	"	"
"	0.068	"	"
"	0.0676	"	"
		Average 0.188	
Copper acetate	0.068 gm.	$Mg_2P_2O_7=0.19$	per cent phosphorus.
"	"	"	"
"	0.066	"	"
"	0.066	"	"
"	0.069	"	"
		Average 0.190	

The results show that about 25 percent of oxidation products are formed when dissolving the phosphorus in the alcohol.

It may be stated that the spirit under examination was prepared with ordinary 95 percent alcohol, the boiling being continued for 12 hours. Upon standing the spirit became cloudy very rapidly and a yellow precipitate was formed.

When applying the copper nitrate method on the same sample of spirit kept at ordinary temperature in small, completely filled bottles at intervals of one month the following results were obtained:

Date of Assay, September 26, 1914.

.0505 gm.	Mg ₂ P ₂ O ₇	=0.1409	per cent	elementary phosphorus.
.0500 "	"	=0.1395	"	" " "
.0508 "	"	=0.1418	"	" " "
.0650 "	"	=0.1813	per cent	total phosphorus estimated directly.
.0680 "	"	=0.1897	"	" " "
.0680 "	"	=0.1897	"	" " "
.0676 "	"	=0.1886	"	" " "

Practically no change within one month.

October 26, 1914

<i>Elementary Phosphorus</i>				<i>Oxidation Products</i>	
.0530 gm.	Mg ₂ P ₂ O ₇	0.1479	per cent	.0106 gm.	Mg ₂ P ₂ O ₇ .0296 per cent
.050 "	"	0.1395	"	.0086 "	" .0240 "

Estimated Directly

.063 gm.	Mg ₂ P ₂ O ₇	0.1758	per cent
.058 "	"	0.1618	"

November 23, 1914

.052 gm.	Mg ₂ P ₂ O ₇	0.1415	per cent	.007 gm.	Mg ₂ P ₂ O ₇ .0195 per cent
.053 "	"	0.1479	"	.006 "	" .0167 "
.0464 "	"	0.1294	"	.005 "	" .0139 "
.0460 "	"	0.1283	"	.004 "	" .0112 "

December 29, 1914

.0477 gm.	Mg ₂ P ₂ O ₇	0.1327	per cent	.0041 gm.	Mg ₂ P ₂ O ₇ .0117 per cent
.049 "	"	0.1367	"	.004 "	" .0112 "

January 25, 1915

.0476 gm.	Mg ₂ P ₂ O ₇	0.1328	per cent	.004 gm.	Mg ₂ P ₂ O ₇ .0112 per cent
.0486 "	"	0.1356	"	.003 "	" .0084 "

February 28, 1915

.0468 gm.	Mg ₂ P ₂ O ₇	0.1307	per cent	.0060 gm.	Mg ₂ P ₂ O ₇ .016 per cent
.0472 "	"	0.1318	"	.0070 "	" .0195 "

March 29, 1915

.0472 gm.	Mg ₂ P ₂ O ₇	0.1318	per cent	.012 gm.	Mg ₂ P ₂ O ₇ .0335 per cent
.0480 "	"	0.1340	"		

May 3, 1915

.048 gm.	Mg ₂ P ₂ O ₇	0.134	per cent	.0105 gm.	Mg ₂ P ₂ O ₇ .0293 per cent
.048 "	"	0.134	"	.0105 "	" .0293 "

June 7, 1915

.047 gm.	Mg ₂ P ₂ O ₇	0.1312	per cent	.004 gm.	Mg ₂ P ₂ O ₇ .0112 per cent
.046 "	"	0.1284	"	.005 "	" .0139 "

In order to find out whether or not spirit of phosphorus when kept in completely filled bottles in a refrigerator retains the elementary phosphorus still better the following experiments were undertaken:

October 27, 1914

<i>Elementary Phosphorus</i>				<i>Oxidation Products</i>	
.049 gm.	Mg ₂ P ₂ O ₇	0.1368	per cent	.009 gm.	Mg ₂ P ₂ O ₇ .0251 per cent
.048 "	"	0.1339	"	.0064 "	" .0179 "
Total phosphorus .056 gm. Mg ₂ P ₂ O ₇ .1536 per cent phosphorus					

November 24, 1914

.041 gm.	Mg ₂ P ₂ O ₇	.1144	per cent	.003 gm.	Mg ₂ P ₂ O ₇ .0084 per cent
.044 "	"	.1221	"	.007 "	" .0195 "
.042 "	"	.1171	"	.004 "	" .0112 "

December 29, 1914			
<i>Elementary Phosphorus</i>		<i>Oxidation Products</i>	
.035 gm.	$Mg_2P_2O_7$.0977 per cent	.005 gm. $Mg_2P_2O_7$.0139 per cent
.037 "	"	.1032 "	.005 " " .0139 "
.037 "	"	.1032 "	.005 " " .0139 "
.035 "	"	.0977 "	.0044 " " .0132 "
January 26, 1915			
.033 gm.	$Mg_2P_2O_7$.0921 per cent	.006 gm. $Mg_2P_2O_7$.0167 per cent
.033 "	"	.0921 "	.004 " " .0112 "
.0332 "	"	.0927 "	.004 " " .0112 "
.033 "	"	.0921 "	.004 " " .0112 "
February 3, 1915			
.034 gm.	$Mg_2P_2O_7$.0949 per cent	.015 gm. $Mg_2P_2O_7$.0293 per cent
.033 "	"	.0921 "	.0115 " " .0321 "
.033 "	"	.0921 "	.006 " " .0167 "
.034 "	"	.0949 "	.008 " " .0223 "
March 30, 1915			
.033 gm.	$Mg_2P_2O_7$	0.921 per cent	.009 gm. $Mg_2P_2O_7$.0251 per cent
.033 "	"	0.921 "	.010 " " .0279 "
.0364 "	"	0.1016 "	.007 " " .0195 "
May 7, 1915			
.047 gm.	$Mg_2P_2O_7$.131 per cent	.0055 gm. $Mg_2P_2O_7$.0154 per cent
.046 "	"	.128 "	.0040 " " .0111 "
.047 "	"	.131 "	.006 " " .0167 "
.046 "	"	.131 "	.005 " " .0130 "
June 11, 1915			
.029 gm.	$Mg_2P_2O_7$.0810 per cent	.006 gm. $Mg_2P_2O_7$.0167 per cent
.028 "	"	.0782 "	.005 " " .0130 "
.031 "	"	.0866 "	.004 " " .0111 "
.030 "	"	.0838 "	.004 " " .0111 "

These results show that spirit of phosphorus when kept in completely filled bottles, protected from light, either at ordinary temperature or in a refrigerator keeps fairly well; they also show that at times a somewhat more rapid deterioration takes place, as explained by comparing results obtained in February and March with those obtained in May. We are at present not in a position to say to what causes such a deterioration can be attributed.

That, however, spirit of phosphorus should be kept in completely filled, preferably amber, bottles is shown by the following experiment. About 300 cc. of spirit was left in a 2500 cc. flint bottle. The spirit which was at first slightly turbid, soon became transparent and after the lapse of about three weeks all the elementary phosphorus had disappeared or in other words had been oxidized.

STABILITY OF PHOSPHORUS PILLS.

The phosphorus pills used for the examination were made from a paste calculated to contain 10 percent of phosphorus and this paste when assayed according to the following process gave the results indicated below.

About .6 gm. of the paste was mixed in a bottle filled with carbonic acid gas with 20 cc. of water and 50 cc. of chloroform both air-free and the mixture

was shaken for about one-half hour. Tragacanth was then added, the mixture shaken again and, after clearing, an aliquot part of the chloroformic solution was treated with copper nitrate in the regular way. The following results were obtained:

.688 gm. of paste gave	.245 gm.	$Mg_2P_2O_7$	=9.94 per cent
.418 " " " "	.145 " "	" "	=9.9 " "
.562 " " " "	.198 " "	" "	=9.85 " "
.423 " " " "	.1466 " "	" "	=9.68 " "

For making the pills a 10 percent excess of phosphorus paste was added to the calculated amount, in order to meet the deterioration or volatilization which occur during the manufacturing process. The pills were assayed strictly according to the process given in our previous paper.

Phosphorus Pills 1/50 gr. Coated.

Date of Assay—		gm. $Mg_2P_2O_7$		per cent
October 2, 1914.....	0.02024	gm.	$Mg_2P_2O_7$	101.2
	0.02110	"	"	105.5
	0.02116	"	"	105.8
November 2, 1914.....	0.02007	"	"	100.35
	0.02078	"	"	103.90
	0.02065	"	"	103.25
December 1, 1914.....	0.02039	"	"	101.95
	0.02060	"	"	103.00
January 7, 1915.....	0.01993	"	"	99.65
February 26, 1915.....	0.02031	"	"	101.55
	0.02038	"	"	101.90
March 4, 1915.....	0.0211	"	"	105.5
April 1, 1915.....	0.0198	"	"	99.0
	0.0205	"	"	102.5
May 11, 1915.....	0.0202	"	"	101.0
June 15, 1915.....	0.01946	"	"	97.3
	0.01969	"	"	97.45

Phosphorus Pills 1/50 gr. Uncoated.

Date of Assay—		gm. $Mg_2P_2O_7$		per cent
September 30, 1914.....	0.02073	gm.	$Mg_2P_2O_7$	103.65
	0.01957	"	"	97.85
November 4, 1914.....	0.02031	"	"	101.55
	0.02073	"	"	103.65
	0.02053	"	"	102.65
December 1, 1914.....	0.02046	"	"	102.30
	0.02075	"	"	103.75
January 6, 1915.....	0.02013	"	"	100.65
	0.02059	"	"	102.95
February 26, 1915.....	0.2005	"	"	100.25
March 4, 1915.....	0.019	"	"	98.5
	0.0205	"	"	102.5
April 1, 1915.....	0.0199	"	"	99.5
	0.0206	"	"	103.00
May 11, 1915.....	0.0199	"	"	99.5
June 15, 1915.....	0.01964	"	"	98.2

Phosphorus Pills 1/100. Coated.

Date of Assay—					
October 2, 1914.....	0.01054	gm. $Mg_2P_2O_7$	105.4	per cent	
	0.01043	" "	104.3	"	
November 2, 1914.....	0.00949	" "	94.9	"	
	0.00942	" "	94.2	"	
	0.01020	" "	102.0	"	
December 1, 1914.....	0.01005	" "	100.0	"	
	0.009908	" "	99.08	"	
January 6, 1915.....	0.01014	" "	101.4	"	
	0.01002	" "	100.2	"	
June 15, 1915.....	0.00978	" "	97.8	"	
	0.00997	" "	99.7	"	

Phosphorus Pills 1/100 gr. Uncoated.

Date of Assay—					
September 30, 1914.....	0.01009	gm. $Mg_2P_2O_7$	100.9	per cent	
	0.01036	" "	103.6	"	
November 2, 1914.....	0.00996	" "	99.6	"	
	0.01017	" "	101.7	"	
December 1, 1914.....	0.01310	" "	103.1	"	
	0.01021	" "	102.1	"	
January 6, 1915.....	0.01002	" "	100.2	"	
	0.01013	" "	101.3	"	
June 15, 1915.....	0.00956	" "	95.6	"	

Phosphorus, Nux Vomica and Damiana Pills, both plain and gelatin coated were then examined. Each pill originally contained 1/100 gr. of phosphorus. The following results were obtained:

Phosphorus, Nux Vomica and Damiana Pills. Uncoated.

Date of Assay—					
December 4, 1914.....	.008926	gm. $Mg_2P_2O_7$	89.26	per cent	
	.009007	" "	90.07	"	
June 16, 1915.....	.00843	" "	84.3	"	
	.00832	" "	83.2	"	

Phosphorus, Nux Vomica and Damiana Pills. Coated.

Date of Assay—					
November 11, 1914.....	.009342	gm. $Mg_2P_2O_7$	93.42	per cent	
	.009491	" "	94.91	"	
December 4, 1914.....	.009084	" "	90.84	"	
June 16, 1915.....	.00808	" "	80.8	"	
	.00817	" "	81.7	"	

Although 10 per cent more phosphorus had been added to the pill mass than theoretically required, the pills assayed below the required amount of phosphorus, thus showing that a part of the phosphorus is oxidized during the manufacturing process. It is further shown that a portion of the phosphorus is oxidized on keeping the pills, no matter whether they are coated or uncoated.

The assay of the pills was carried out according to the process outlined in our previous paper with the exception that the shaking of the chloroformic phosphorus solution with the copper nitrate solution was continued for three hours or until the green color of the mixture had acquired a brownish tint due to the formation of copper phosphide. We have found that certain vegetable

matters retard the formation of copper phosphide considerably and unless all the phosphorus has been converted into phosphide the phosphorus cannot be converted into its oxidation products by hydrogen peroxide but will remain as such and appear in the bottle in the form of white fumes.

We also had this experience when determining:

STABILITY OF ELIXIR OF PHOSPHORUS, NUX VOMICA AND DAMIANA.

The elixir was prepared from a spirit of phosphorus which assayed 0.1224 per cent of phosphorus. 280 cc. of this spirit was mixed with 720 cc. of elixir of nux vomica and damiana. The elixir thus prepared should contain theoretically 0.035 per cent of phosphorus, viz.: four times the amount required by the product generally found on the market. The elixir was kept in small completely filled amber bottles, protected from light and at ordinary temperature.

Assays of the Elixir.

Date of Assay—

November 17, 1914.....	.026	gm. Mg ₂ P ₂ O ₇	.02905	per cent
	.024	“ “	.02681	“
	.026	“ “	.02905	“
December 5, 1914.....	.024	“ “	.02681	“
	.025	“ “	.02794	“
January 5, 1915.....	.021	“ “	.02346	“
	.0214	“ “	.02391	“
January 30, 1915.....	.020	“ “	.02235	“
	.021	“ “	.02346	“
March 15, 1915.....	.017	“ “	.01864	“
	.021	“ “	.02346	“
April 1, 1915.....	.017	“ “	.01864	“
May 10, 1915.....	.011	“ “	.01228	“
	.0118	“ “	.01264	“
June 14, 1915.....	.008	“ “	.0892	“
	.010	“ “	.01116	“

These results show that elixir of phosphorus, nux vomica, and damiana keeps well for about five months, after which time a rather rapid dissipation of the yellow phosphorus takes place.

In summing up we find that preparations containing yellow phosphorus keep fairly well under ordinary conditions. Only those preparations which contain vegetable matter are easily decomposed.

RESEARCH LABORATORY OF SHARP & DOHME.

REMARKS AND DISCUSSIONS.

Dr. Turner: I merely wish to compliment Dr. Engelhardt on his paper and to point out that the question of determining phosphorus in compounds is a very complicated one. Now we have something which will really enable us to determine the amount of phosphorus in these preparations.

Dr. Dohme: I would like to ask Dr. Engelhardt to what extent the results agree with one another in the various determinations.

Dr. Engelhardt: The experiments were made in duplicate and triplicate and the results vary, I should judge, within the limit of 3 per cent. or 5 per cent. Often the variation was even less. In my opinion the results are particularly satisfactory because with other processes the results obtained were far from being concordant, and in some cases, were absolutely unreliable. I believe we have tried all processes recommended up to the present time

for estimating yellow phosphorus in pharmaceutical preparations and especially the process requiring the destruction of the organic matter and oxidation of the phosphorus by the Kjeldahl method. The fact that the results obtained by this method were not at all concordant may be due to a too rapid oxidation of the phosphorus which manifests itself at times by small explosions.

We have found that with the method outlined by us we can get uniform and satisfactory results and when you get results which remain within the 5 per cent limit and taking even the personal equation into consideration within the 8 per cent limit you should be very well satisfied.

A Member: Are these phosphorus compounds, that is, the therapeutic products, pills, etc., used as extensively as they were some years ago? What is the demand?

Dr. Engelhardt: My impression is that we still make rather large quantities of pharmaceutical preparations containing phosphorus, especially those containing in addition to phosphorus, extract of nux vomica and extract of damiana. On account of the instability of phosphorus in elixir of phosphorus, nux vomica and damiana we have quite recently replaced the phosphorus by glycerophosphates. Such a preparation is apparently equally as effective, for we continue to have a great demand for it.

A Member: It is perhaps not germane to the subject, but I should like to ask whether there is any real evidence as to the therapeutic value of either elixir of phosphorus or glycerophosphates?

Dr. Engelhardt: You had better ask the American Medical Association. They claim not.

SEMPERVIRINE FROM GELSEMIUM ROOT*.

A. E. STEVENSON AND L. E. SAYRE.

The authors give a method of separating "Sempervirine" from a mixture of the combined alkaloids of gelsemium root, by means of converting the alkaloid into the nitrate which is quite insoluble in water and practically insoluble in solution containing sodium nitrate. Methods of preparing the free alkaloid and some of its salts, a description of these products, and their reactions with the usual alkaloidal reagents are given. In the second part of the paper the authors describe gelseminine which, they claim, consists of at least two alkaloids. They further suggest a method for separation of the various alkaloids of the root and the results of some preliminary physiological experiments carried out with sempervirine, which have shown that the salt apparently has no *immediate* toxic effect, but its definite toxicity is stated.—(Editor.)

At previous annual meetings of this Association, the progress in the investigation of Gelsemium Root has been contributed. In former papers, the various contributions of different authors have been referred to and, at this time, it is unnecessary to repeat the same. It is the desire of the present authors to record in the proceedings of this Association any results of this investigation.

In the present paper, we desire to report further progress in the separation of the various alkaloids of this interesting drug and will confine ourselves very briefly to the method of separation of the alkaloid sempervirine from the total alkaloids of Gelsemium. This report, taken in connection with former

*Presented to Scientific Section, A. Ph. A., San Francisco.