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Spectro-Chemical Studies on Some Biochemical Color Reactions.

By

Tetsutaro Tadokoro.

With 31 Figures and 20 Photograms.

There are many color reactions commonly used in the biochemical investigations of natural stuff. But in these investigations, we meet often with the difficulty of distinguishing the true color reaction from reaction caused by the presence of contaminating colors. For the distinction of the true color from a similar color, or for the sifting of the true one from contaminating colors, spectrochemical methods are very important because they are proved to be exact for the detection of the true color reaction.

Until now, many spectrochemical results (to which we refer in the following experimental parts) have been reported, but almost all measurments are inaccurate. Most of them were measured only in the visible portion and there is no result given as to the absorption band in the ultraviolet portion of the spectrum. The following spectrochemical investigations of color-reactions were undertaken to obtain accurate measurements of absorption bands with a quartz spectroscope. As a source of light we used an electric lamp for the visible portion and an iron arc or a hydrogen lamp for the invisible portion of the spectrum.

[Jour. of the College of Agric., Hokkaido Imp. Univ., Sapporo, Vol. X., Pt. 6, Dec. 1922.]

EXPERIMENTS.

In the following experiments we used as a source of light an electric lamp, an iron arc and a hydrogen tube with a capillary 5 cm. long, filled with hydrogen at 8 mm. pressure. For the operation of this hydrogen lamp, we used an alternating high voltage current obtained from a transformer. The primary circuit was supplied with alternating curreut at 220 volts, and a rheostat in series was used to maintain the primary current at four amperes, giving a voltage of 2000 in secondary. A quartz cell 12 mm. thick was filled with the mixture of reagent and interposed between the hydrogen lamp and the spectrograph. Here all photographs were taken with 7 minutes exposure, using panchromatic Wratten and Wainright dry plates. The method of obtaining a uniform ultraviolet spectrum by the use of hydrogen tube was described by Prof. H. B. LEMON[1] and we also used the same light source.

As a source of light for the visible portion of spectrum, we used a tungsten lamp of 100 candle power and as farther source of light for the invisible portion, an iron arc was prepared with 250 volts and 4 amperes, direct current. Photographs were taken with 6 seconds exposure, using the iron arc and with 30 seconds exposure by the tungsten lamp.

SAMPLES.

Glucose...(f. Karlbaum)...white crystalline powder...m.p. 146°·C...$[\alpha_D]=+51°,9$...Birotaton.
Fructose...(„)...light brown crystal...m.p....94°·5C...$[\alpha_D]=-91°·5$...very hydroscopic.
Mannose...(„)...white powder...m.p. 132·°5C $[\alpha_D]=+14·°5$...forms phenylhydrazon.
Galactose („) ..white crystalline powder...m.p. 165°·5C...$[\alpha_D]=+80·°9$ forms music acid.
Arabinose („)...white crystalline powder...m.p. 159·°C....$[\alpha_D]=+105°$...forms furfurol.
Xylose... („)... „ „ „ m.p. 151·°C....$[\alpha_D]=+18°,5$ „
Furfurol (by author) light yellow liquid...B.p. 162°C...sp. gr.=1·1628.
Creatinin (by prof. Koch, Chicago, Univ., U.S.A.)...prismatic crystal...m.p. 234°C...forms 1,6 times of zinc chloride salt.
Tryptophan („)...silky crystal...m.p. 252°·C...$[\alpha_D]=-32·°5$.
Tyrosin (f. Karlbaum)...neadle like crystal...m.p. 298,°C...$[\alpha_D]=-8·°5$.
Histidin („) plate like crystal...m.p. 252°·C...$[\alpha_D]=-6·°5$...positive pyrrol reaction.
Uric acid („)...rhombic crystal...positive Deniges and Schiff's murexid reaction.
Skatole (by Prof. Koch)...brownish plate like crystal...m.p. 93·°C...faecal odor.
Arbutin (f. Karlbaum) silky crystal...m.p. 147°·C...no reducing power.
Gallotannic acid („)...yellow powder...m.p. 153°·C. (carbonify) 209°·C. (decomposed).
Salicylic acid („) Needle like crystal...m.p. 155·°5C...soluble chloroform and ether.
Antipyrin (f. Park Devis) crystalline powder...m.p. 111·°5C...precipitate with tannic acid.

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1) Lemon:—Astrophysical Journal, XXXV., 109–124 (1922).

Quinin Hydrochloride („) white crystalline powder...m.p. 185.°5C...green color with "CL" water and ammonia, soluble in chloroform.
Codein phosphate („) prismatic crystalline powder...m.p. 152°.5 C...colorless in H_2SO_4.
Strychnin („) colorless rhombic crystal...m.p. 265.°C...soluble in chloroform.
Nicotin (Karlbaum) yellowish brown liquid. $[\alpha_D] = -160.°8$...soluble in ether and alcohol.
Morphin (Park Devis) rhombic crystal...m.p. 228.°C...laevo-rotation.

Part I. Some color reactions of Sugars.

A. **Hexose.**

1. NEUMAN's orcin reaction :—Orcin reaction[1] is used for the detection of hexose as follows: when one drop of acetic acid and of orcin alcohol solution are added to hexose solution and heated with sulphuric acid, the hexose shows a reddish brown color. Now we examined this color reaction using a spectroscope with an electric lamp and an iron arc.

TABLE I.

Concentration of sugar sol.	Reagents	Wave-length of a boundary line of the absorption band	
		Glucose	Fructose
Electric lamp	5cc H_2O+1 drop orcin acetic acid+5cc H_2SO_4	3490	.....
1/8 %	(5cc) of sample „ „	5080—3820	3820
1/4 %	„ „ „	5150—5050—3875	5250
1/2 %	„ „ „	5175—5000—5890	5650
1 %	„ „ „	5225—4975—3915	6105
1.5%	„ „ „	5275—4975—3990	.....
Remarks		„	most intense

Here we trace the following curves from the above results, putting the log. of thickness mm. of $\frac{\text{mol.}}{10000}$ solution on the ordinate and the wave length on the abscissa.

1) Zs. physiol. Chem. Bd., **12**, 355, (1888).

Fig. 1. (See Phot. A.)

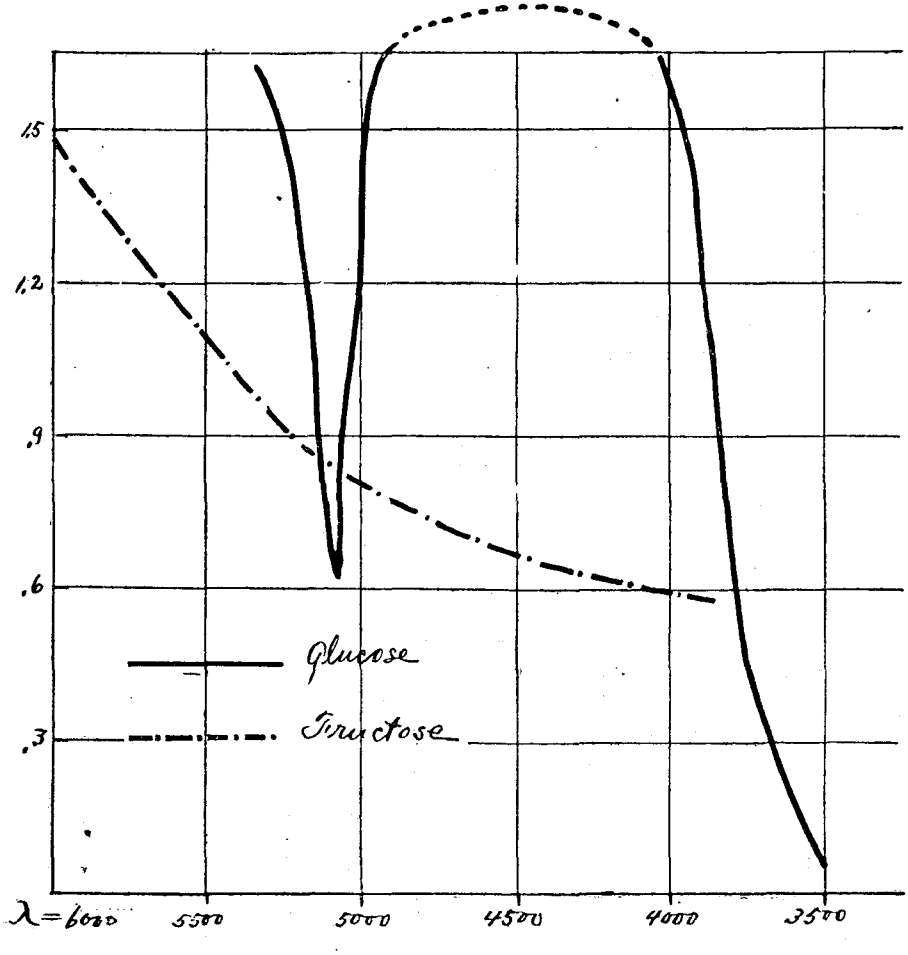
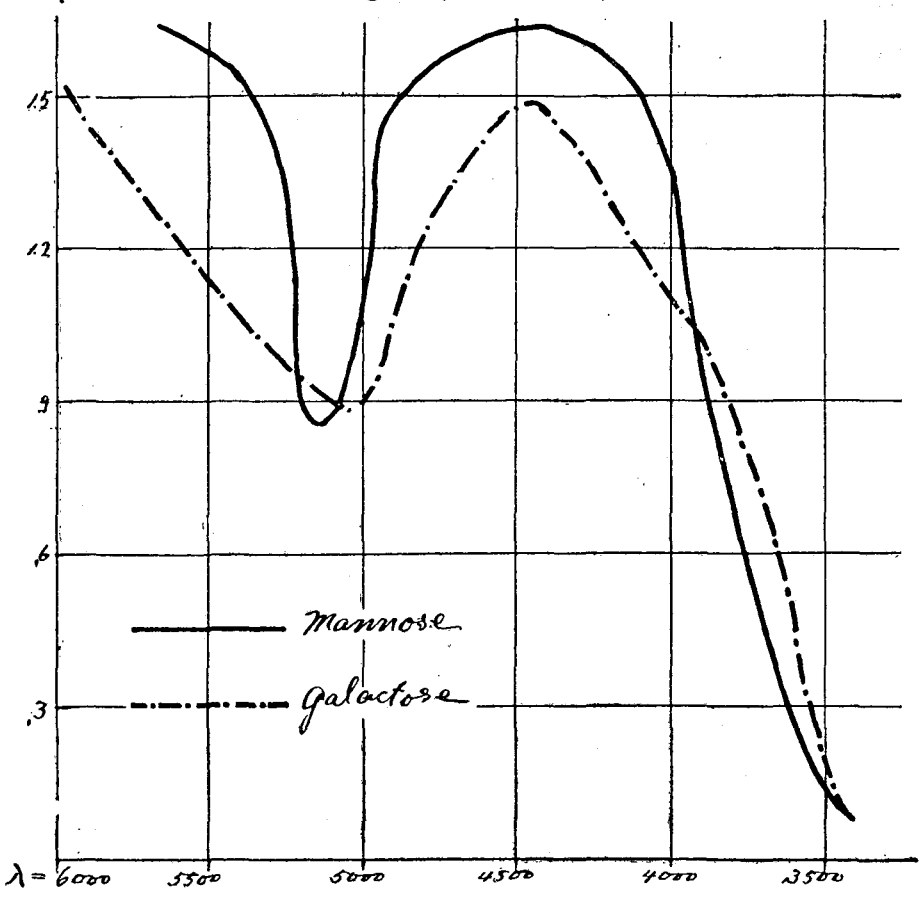


TABLE 2.

Concentration of sugar sol.	Reagents	Wave-length of a boundary line of the absorption band	
		Mannose	Galactose
Electric lamp	5cc H_2O+1 drop orcin acetic acid+5cc H_2SO_4	3330	3305
1/8 %	5cc of sample " "	3775	3675
1/4 %	" " "	5200—5050—3845	5095—4975—3760
1/2 %	" " "	5250—4975—3965	5575—4825—4150
1 %	" " "	5300—4925—4050	5950—4400
1.5 %	" " "	5575—4475	——
Remarks		intense	intense

Here we trace the following curves from the above results, with the same treatment as above.

Fig. 2. (See Phot. B.)



From these results we observed that the color reactions of glucose, mannose and galactose have two absorption bands between 5000–5300 and 3500–4000$\mu\mu$. The former is the most typical band and shows the sharp boundary edge, but the latter is not so sharp and its boundary line grows in the direction of longer wave lengths from 3500$\mu\mu$. But fructose shows no typical band between 5000–5300$\mu\mu$ and the boundary line grows gradually in the direction of longer wave lengths from 3500$\mu\mu$. Comparing the absorption bands of four sugars, the difference of their characters is as follows:—

TABLE 3.

	Characters	Glucose	Fructose	Mannose	Galactose
Band betw. 5000–5300	Edge of the boundary line	very sharp	no band	sharp	dull
	Form of band	very narrow	.....	narrow	wide
	Absorption appear from concentration	1/8%	.....	1/4%	1·4%
	Color reaction	very sensitive	.....	sensitive	sensitive
3500–4000	Band grows.....	very slowly	very rapidly	slowly	rapidly
	Band fused with the former in the concentration of stronger than	1·5%	No definite point, fused gradually all along the boundary line.	1 %	1/2%

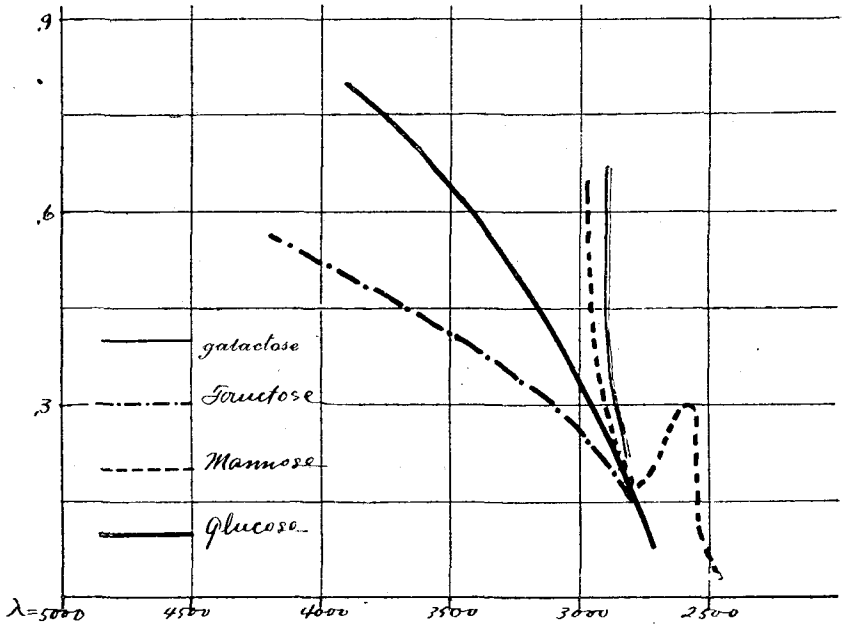
TABLE 4.

Concentration of sugar sol.	Reagents	Wave-length of a boundary line of the absorption band			
		Glucose	Fructose	Mannose	Galactose
Iron arc	5cc H_2O+1 drop orcin acetic acid+5cc H_2SO_4	2160	——	2280	——
1/32%	5cc of sample "	2825	2852	2855–2550	2855
1/16%	" "	2875	3125	2865–2560	2985
1/8%	" "	2925	5175	2965	3425
1/4%	" "	——	5170	——	4474
Remarks	Band grows "	very slowly	very rapidly	slowly	rapidly

From above results, we observed that mannose has a typical absorption band in the ultra portion between 2500–3000$\mu\mu$., but glucose, fructose and galactose have no typical band only grow gradually in the direction of longer wave lengths.

Here we trace the following curves from the above results, with the same treatment as above.

Fig. 3.



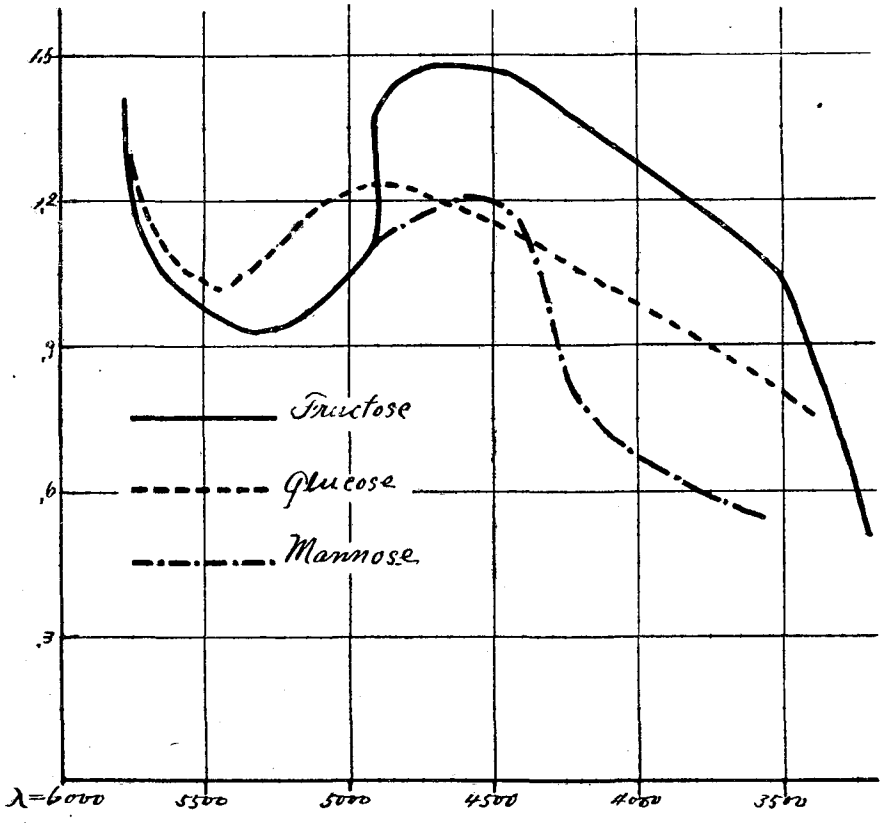
2. Diazobenzenesulphonic acid reaction of hexose was examined with sodium amalgam in the presence of alkalies, using an ultra-spectroscope and the following results were obtained.

TABLE 5.

Concentration of sugars sol.	Reagents	Wave-length of a boundry line of the absorption bands		
		Glucose	Fructose	Mannose
Iron arc	5cc H_2O+1 drop diaz. +2cc Alkali sol.	.....	3010	.....
1/8%	5cc of sample „ „	3180	3215	.....
1/4%	„ „ „	3445	3425	4225
1/2%	„ „ „	5700–5275–4350	5725–4900–3510	5725–4900–4350
1 %	„ „ „	5775	5775–4900–4350	.....
Remarks	Color reaction	intense	.....	very intense

Here we trace the following curves from the above results.

Fig. 4.



From the above results we observe that these three sugars have a very similar absorption band in the portion between 4800–5750$\mu\mu$, but not in the portion between 3500–4500$\mu\mu$.

B. **Pentose.**

3. Phloroglucin reaction:—Pentose solution reacts with phloroglucin when boiled with the same volume of strong HCL and gives a color reaction. We examined this reaction with xylose and arabinose, using an ultra-spectroscope [1]

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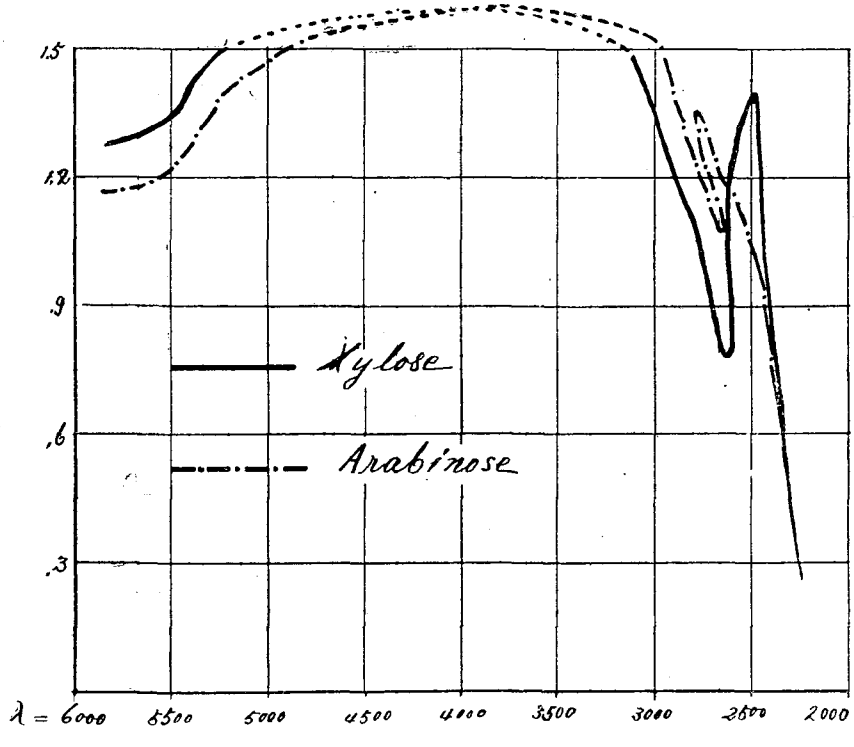
1) Ihl:—Chemiker Ztg. Jg. S. 231 (1885).
Pinoff:—Ber. deuts. chem. Ges. 38, 766 (1905).
Bial:—Biochem. Zs. 3, 323 (1907).

TABLE 6.

Construction of sugar solution	Reagents	Wave-length of a boundary line of the absorption bands	
		Xylose	Arabinose
Iron arc	5cc H_2O+1 drop phlrog. sol.+4cc HCL	2275	2275
1/40 %	5cc of sample „ „	2675–2645–2385	2455
1/20 %	„ „ „	2775–2645–2440	2680–2650 2470
1/16 %	„ „ „	2905–2645–2485	5510–2645–2650–2615
1/10 %	„ „ „	5450–2990–2500	5350–2735–2755
1/8 %	„ „ „	5335–3115	4925–2990
Remarks	Color reaction	very intense	intense

Here we trace the following curves from the above results.

Fig. 5. (Phot. CA.)



In these reactions of two sugars, we observed two very similar absorption bands between 6000–5000$\mu\mu$ and 2600–3200$\mu\mu$. The

absorption bands in ultra-parts are more sensitive than in the visible parts of the spectrum.

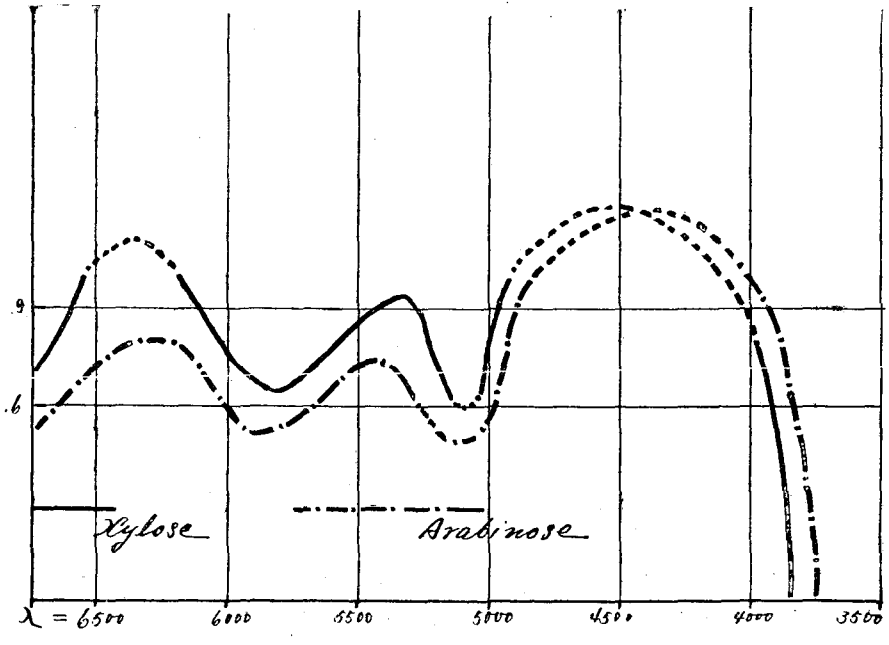
4. NEUMAN's reaction:—This reaction is used to detect the pentose in the same manner as hexose. A violet color results from the use of arabinose and bluish violet color from xylose.

TABLE 7.

Concentration of sugars sol.	Reagents	Wave-length of a boundary line of the absorption band	
		Xylose	Arabinose
Electric lamp	5cc H_2O+1 drop orcin acetic acid+4cc H_2SO_4	5835	5725
1/8 %	5cc of sample ,, ,,	6725–5825–5150–5025 3925	6725–6075–5660–5275 4950–3775
1/4 %	,, ,, ,,	6550–6125–5590–5275 4950–4075	4850–3875
Remarks	Color reaction	intense	very intense

Here we trace the following curves from the above results.

Fig. 6.



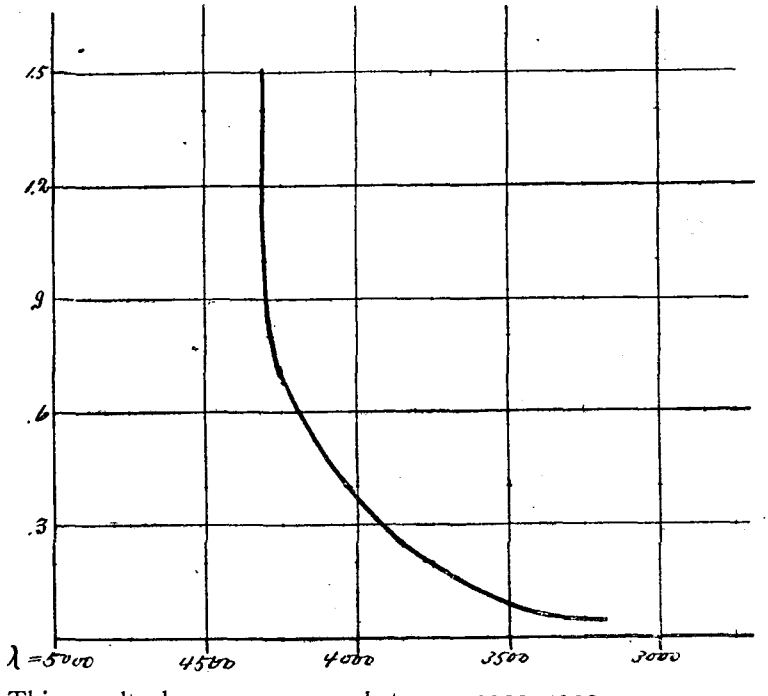
These results showed four absorption bands between 6700–6500$\mu\mu$, 6100–5500$\mu\mu$, 5250–5750$\mu\mu$, and 4000–3500$\mu\mu$ and the two sugars have a similar band, but the intensity of arabinose is stronger than that of xylose.

5. Anilin acetate reaction of furfurol[1]:—The reaction was examined with an ultra-spectroscope and we obtained the following results.

TABLE 8.

Concentration of furfurol sol.	Reagents	Wave-length of a boundary line of the absorption band (Hydrogen lamp)
0.1 %	Furfurol solution only	2300
0.1 %	0.1cc furfurol sol. + 6cc anilin acetate sol.	3050
0.1 %	1.cc "	4295
0.1 %	2.cc "	4295
0.1 %	3.cc "	4295

Fig. 7.



This result shows one curve between 3000–4300$\mu\mu$.

1) See the foot note of Hexose.

Part II. Some color reactions of decomposition products of Protein.

C. **Creatinin.**

6. WEYL's creatinin reaction:—This reaction shows a yellowish red color with sodium nitroprusside and one drop of sodium hydroxide solution.[1] We examined this reaction with an ultra-spectroscope and we obtained the following results.

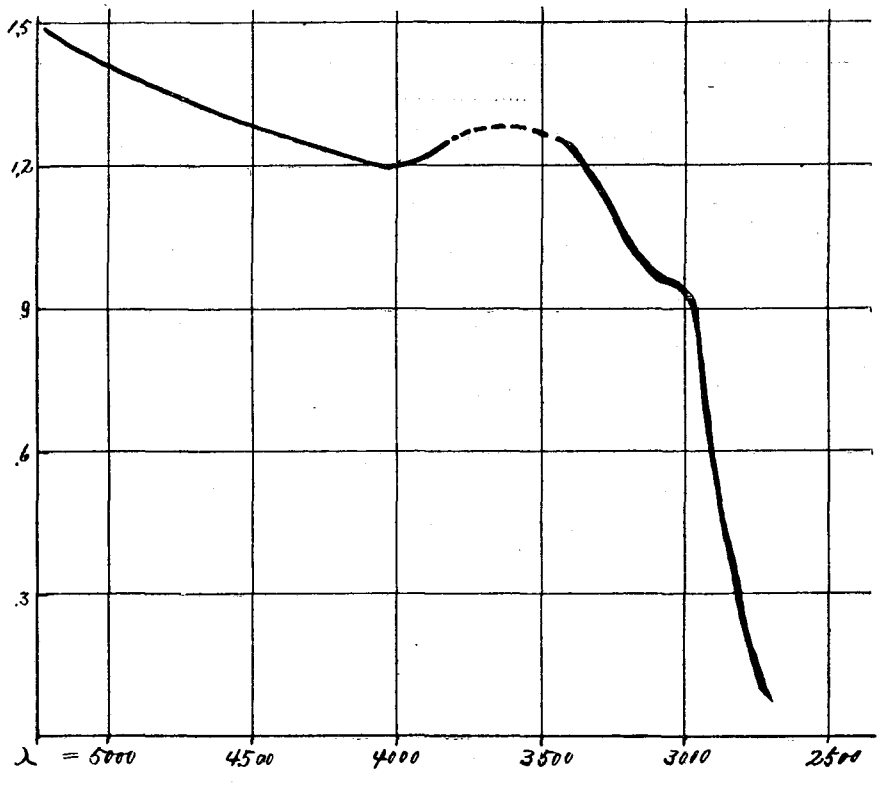
TABLE 9.

Concentration of creatinin solution and reagent	Wave-length of a boundary line of the absorption band
Iron arc. Water only.	2140
8cc H_2O+1cc Na-prusside+0.5cc KOH	2740
8cc 1/16% Creatinin ,, ,,	2740
,, 1/8 ,, ,, ,,	2960
,, 1/4 ,, ,, ,,	2975-3100
,, 1/2 ,, ,, ,,	3485-3855-4245
,, 1 ,, ,, ,,	5475

Here we trace the following curve from the above results.

1) Arnold:—Zs. f. Physiol., 49, 397 (1906).

Fig. 8. (See phot. E.



These results showed us two absorption bands between 4500–3700$\mu\mu$ and 3500–2800$\mu\mu$.

7. JAFFE's reaction :—This creatinin reaction shows a reddish color with picric acid and sodium hydroxide solution.[1] We treated this reaction with an ultraspectroscope as above.

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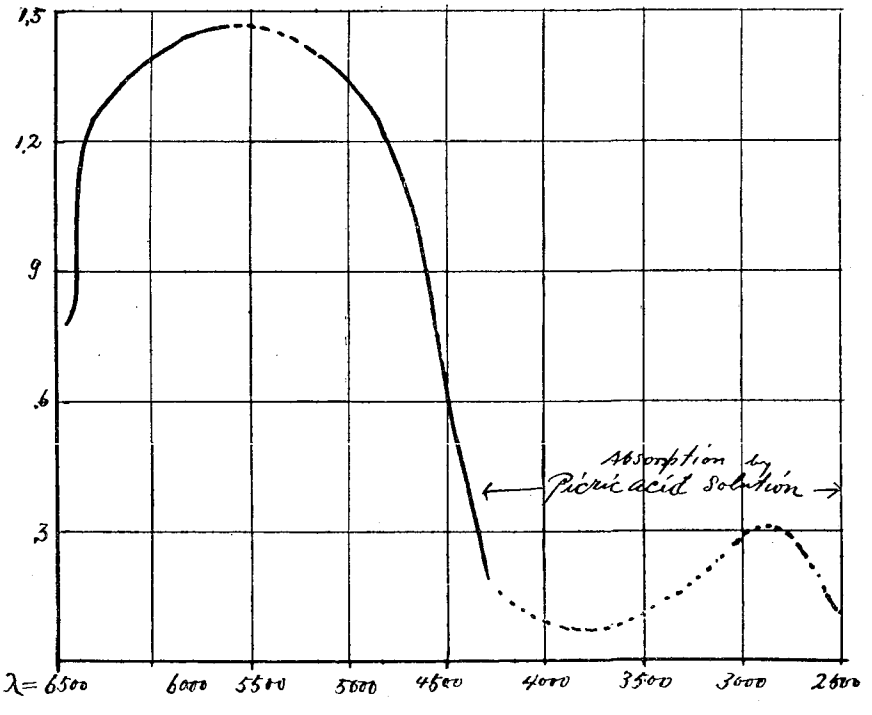
1) Chapman :—Chem. News, 100, 1759 (1909).

TABLE 10.

Concentration of creatinin solution	Reagents	Wave-length of a boundary line of the absorption band
Iron arc	Water only	2140
H_2O	8cc+0.5cc 1% picric acid +0.5cc KOH	2725-3050-4335
1/16 % creat.	8cc " " "	2750-3050-4335
1/4 % "	" " "	4535-6400
1/2 % "	" " "	4800-6370
1 % "	" " "	5260-5850

Here we trace the following curve from the above results.

Fig. 9. (See phot. E.)



These results showed three curves between 7000–6200$\mu\mu$, 4800–4000$\mu\mu$ and 2500–2800$\mu\mu$. The latter (2500–2800) is not the absorption band of creatinin but it belongs to picric acid solution.

D. **Tryptophan.**

8. Reaction with glyoxylic acid:—Tryptophan solution shows a reddish violet color with glyoxylic acid.[1] We examined this reaction with an ultra-spectroscope as above.

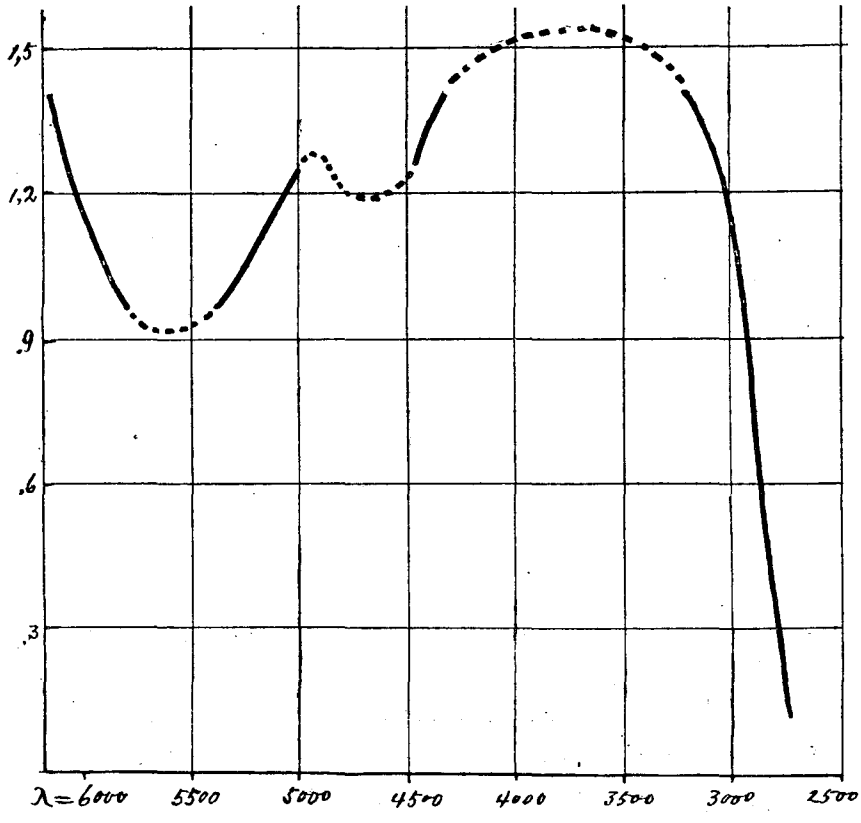
TABLE II.

Concentration of tryptophan solution	Reagents	Wave-length of a boundary line of the absorption band
Iron arc	Water only	2150
H_2O	4cc + 1cc 2% glyoxylic acid + 5cc H_2SO_4	2265
0.02 %	4cc of sample " "	2335
0.05 %	" " "	2735
0.07 %	" " "	2745
0.10 %	" " "	2940
0.25 %	" " "	2965–5400–5800
0.50 %	" " "	2990–4490–4825–4925–6150
0.75 %	" " "	3200–4410–6250

Here we trace the following curve from the above results.

1) Cole:—Jour. of Physiol., 30, 311 (1906).

Fig. 10. (See phot. F.)



This reaction shows three curves between 6250–5000, 5060–4200, and 3400–2800$\mu\mu$.

9. ADAMKIEWICZ's reaction: Tryptophan solution shows a red color by ADAMKIEWICZ's reaction *i.e.* by treating it with acetic acid and strong H_2SO_4. We examined this reaction with an ultraspectroscope and obtained the following results.

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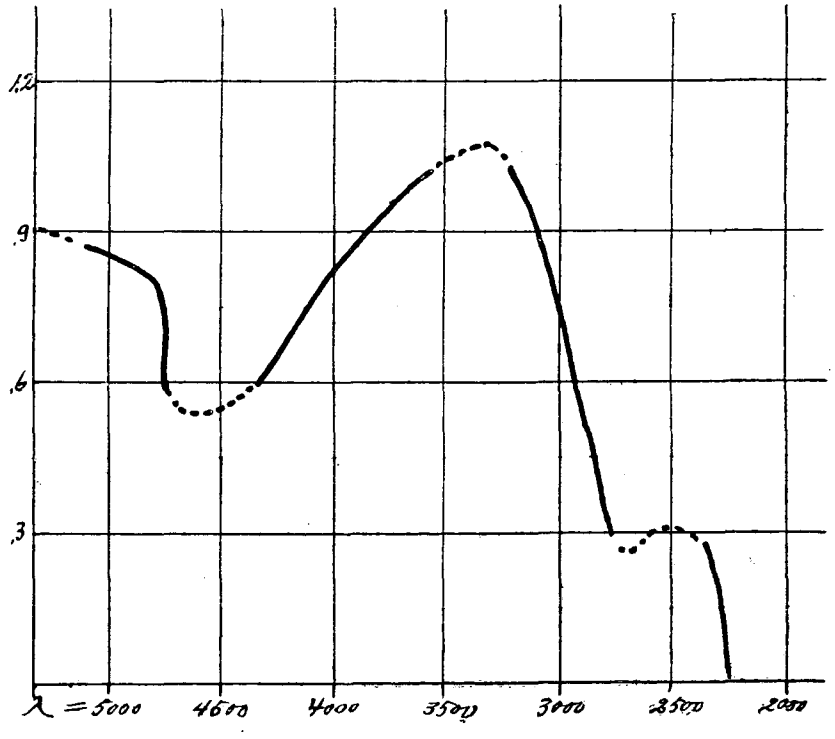
1) Ber. d. dents. chem. Ges., 8, 161 (1875).

TABLE 12.

Concentration of tryptophan	Reagents			Wave-length of a boundary line of the absorption band
Iron arc	H_2O 5cc + 1cc 0·1% acetic acid + 5cc H_2SO_4			2290
0·005 %	5cc of sample	,,	,,	2290
0·025 %	,,	,,	,,	2345
0·05 %	,,	,,	,,	2385–2655–2740
0·075 %	,,	,,	,,	2762
0·10 %	,,	,,	,,	2935
0·125 %	,,	,,	,,	2965–4300–4800
0·15 %	,,	,,	,,	2970–4280–4800
0·25 %	,,	,,	,,	3035–3915–5100
0·30 %	,,	,,	,,	3195–3580

Here we trace the following curve from the above results.

Fig. 11. (See phot. F′.)



The above curve shows two absorption bands between 5200–4000 and 3500–2500$\mu\mu$.

E. **Tyrosin.**

10. WURSTER's reaction:—This reaction of tyrosin shows a red color by adding a few drops 1% of acetic acid and 1% sodium nitrite solution.[1] We examined this reaction with an ultraspectroscope and we obtained the following results.

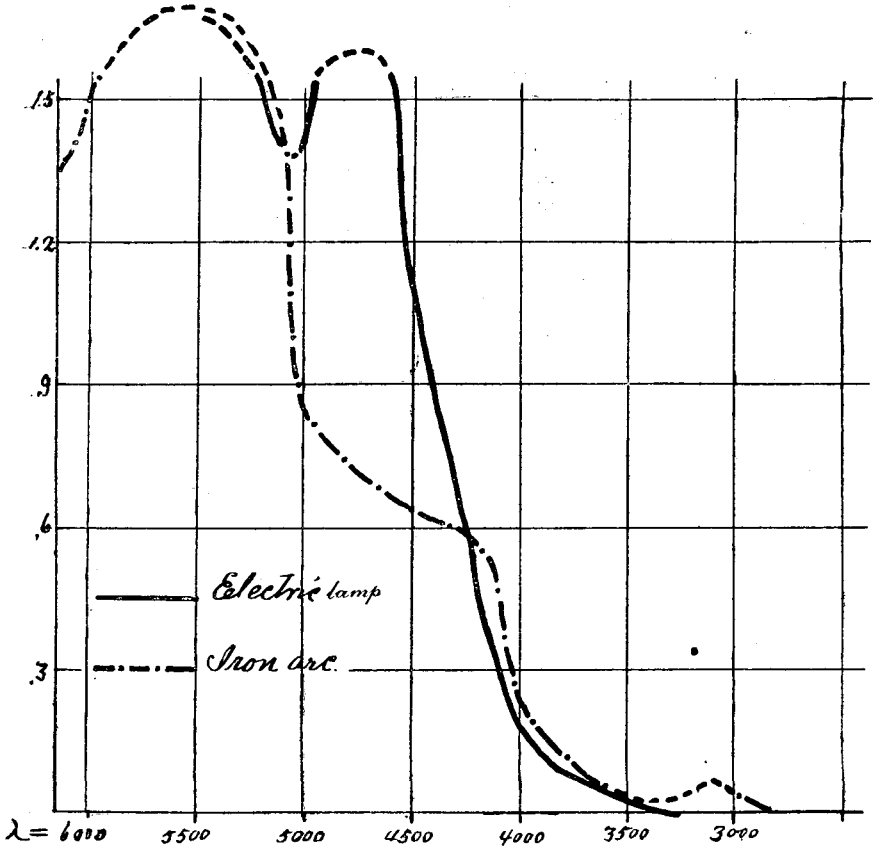
TABLE 13.

Concentration of tyrosin sol.	Reagents	Wave-length of a boundary line of the absorption band	
		Electric lamp	Iron arc
	5cc H_2O+3cc 1% acetic acid +2cc 1% sodium Nitrite	3265	2550
0.02 %	tyrosin sol. 5cc ,, ,,	3765	3010–3280–3680
0.025 %	,, ,, ,,	3935	3775
0.050 %	,, ,, ,,	4085	4000
0.100 %	,, ,, ,,	4275	4145
0.200 %	,, ,, ,,	4395	5075–6600
0.50 %	,, ,, ,,	4550	5075–6275
0.75 %	,, ,, ,,	4550–5175–5050	5075–6150
1.00 %	,, ,, ,,	4550–4950–5225	.....

Here we trace the following curves from the above results.

1) Centr. bl. Physiol., 1, 193 (1888).

Fig. 12. (See phot. G)



From the above results we observed two absorption bands when illuminating with an iron arc between 6000–6500$\mu\mu$ and two bands with an electric lamp between 4800–5500 and 4800–3500$\mu\mu$.

11. Diazo-reaction:—This reaction of tyrosin shows a cherry red color with diazobenzene sulphonic acid and soda solution.[1] We examined this reaction with an ultraspectroscope and we obtained the following results.

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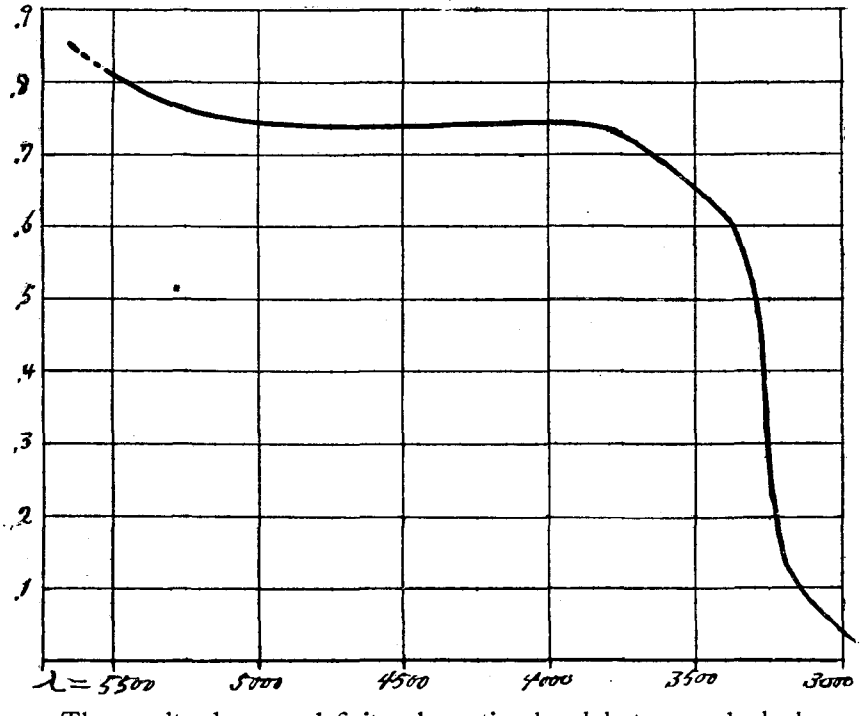
1) Pauly:—Zs. f. physiol. Chem., 42, 517 (1904).

TABLE 14.

Concentration of tyrosin sol.	Reagents	Wave-length of a boundary line of the absorption band (Iron arc.)
H_2O	5cc+1cc diazobenzen sulphonic acid sol+2cc soda sol.	2100
0·5 cc	tyrosin 5/10000+ „ „ „	2170
1·0 cc	„ + 4cc H_2O „	3170
2·0 cc	„ + 3cc „	3270
3·0 cc	„ + 2cc „	3375
4·0 cc	„ + 1cc „	3725
5·0 cc	„ + 0cc „	5525

Here we trace the following curve from the above results.

Fig. 13. (See phot. H.)



The results show no definite absorption band but a gradual absorption of the shorter wave-length to the longer one, *i.e.* 3000–5500$\mu\mu$.

F. **Histidin.**

12. Diazo-reaction:—This reaction of histidin shows a red color with diazobenzenesulphonic acid and soda solution. We examined this reaction with an ultra-spectroscope and obtained the following results.

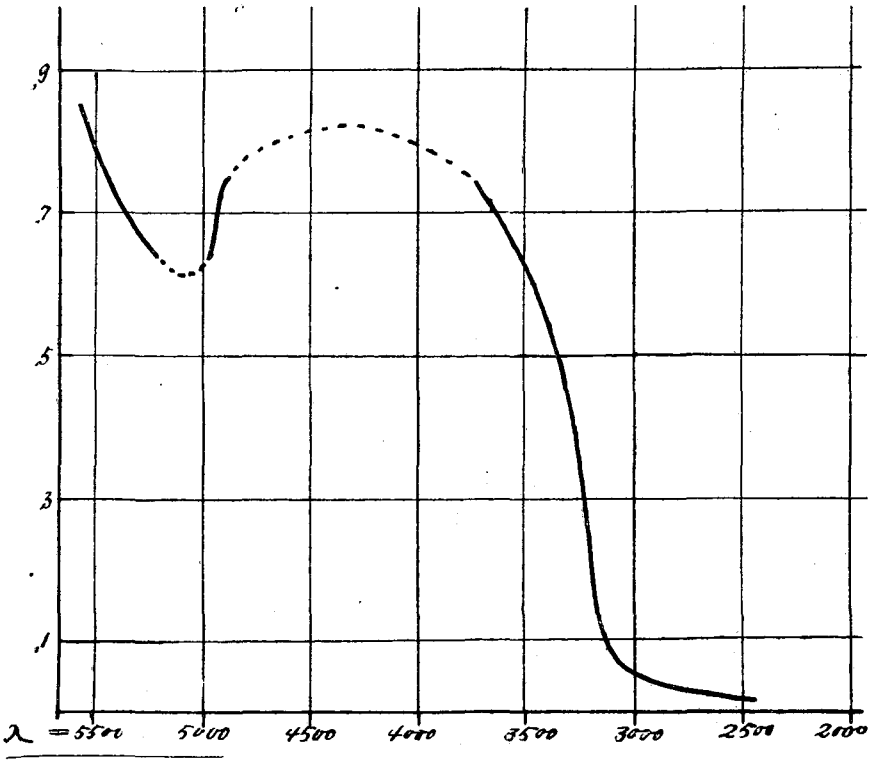
[1]In the following table: A=1/100000 histidin dichloride solution. B=45cc of 4·5% diazobenzenesulphonic acid solution was diluted wtih 500cc of 3% hydrochloric acid solution, C=5% sodium-nitrite, D=1·1% sodium carbonate solution and R=1·5cc of B-solution and 7·5cc of C-solution were diluted to 50cc of water.

TABLE 15.

Concentration of histidin solution and reagents	Wave-length of a boundary line of the absorption band (Iron arc.)
Water only	2100
2cc R+5cc D+3cc H_2O	2170
0·2cc A+2cc R+5cc D+2·8cc H_2O	3170
0·5cc A+2cc R+5cc D+2·5cc H_2O	3270
1·0cc A+2cc R+5cc D+2cc H_2O	3375
1·5cc A+2cc R+5cc D+1·5cc H_2O	5175–4995–3550
2·0cc „ „ 1·0 „	3455–4875–3725
2·5cc „ „ 0·5 „	5525

Here we trace the following curve from the above results.

Fig. 14. (See phot. H.)



[1] Pauly:—Zs. f. physiol. Chem., 42, 508 (1904).

From the above results we obtained two absorption bands between 5500–4800$\mu\mu$ and 2500–3500$\mu\mu$.

G. **Uric acid.**

13. Phosphotungstic acid reaction:—Uric acid solution shows a blue color reaction with phosphotungstic acid and sodium carbonate solution.[1] We examined this reaction with an ultraspectroscope and we obtained the following results.

TABLE 16.

Concentration of uric acid solution and reagents.	Wave-length of a boundary line of the absorption band (Electric lamp)
Iron arc only	2150
Water only	2230
—— 2cc H_2O+3cc 2% tungst. acid +5cc sat. sodium carbonate ...	3135
1cc 5% uric acid+1cc H_2O+ „ „	3335
2cc „ „ „ „ „	3355
3cc „ „ „ „ „	3375

14. K-Bichromate reaction:—Uric acid solution shows a green color reaction with addition of a few drops of strong sulphuric acid and 2% potassium bichromate solution.[2] We examined this reaction with an ultraspectroscope and we obtained the following results.

TABLE 17.

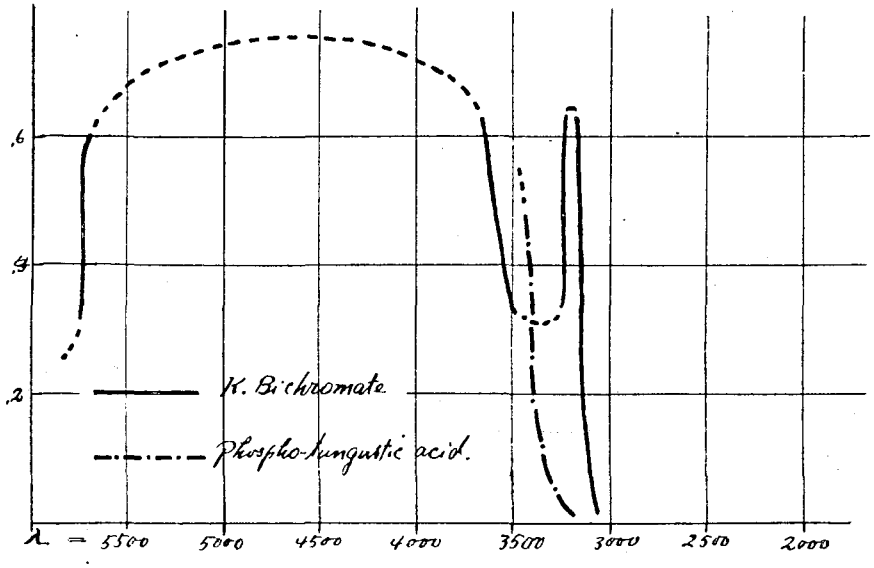
Concentration of uric acid solution and reagents	Wave-length of a boundary line of the absorption band (Iron arc)
Water only (with iron arc)	2230
1cc 2% K-bichromate+1cc str. H_2SO_4+5cc H_2O ...	3055
0.5cc 5% uric acid+1cc 2% K-bichromate+4cc H_2O	3055
1cc 5% „ „ „ 4cc H_2O	3140–3225–3485–5735
2cc „ „ „ 3cc H_2O	3170–3200–3665–5725

Here we trace the following curves from the above two experimental results.

1) Folin:—Jour. Biol. Chem., 13, 363, 1912.

2) Agulhon:—Bull. de la soc. chim. de France, 4, 11, 69 (1912).

Fig. 15.



With the phosphotungstic acid reaction, we observed a band between 3000–3500$\mu\mu$, and with the potassium bichromate reaction, we observed three bands between 6000–5000, 3200–3600 and 3200–3000$\mu\mu$.

H. **Scatol**.

15. P-dimethylbenzaldehyd reaction:—Scatol solution shows a yellow color reaction with a few cc of p-dimethylbenzaldehyd alcohol solution and hydrochloric acid.[1] We examined this reaction with an ultraspectroscope and obtained the following results.

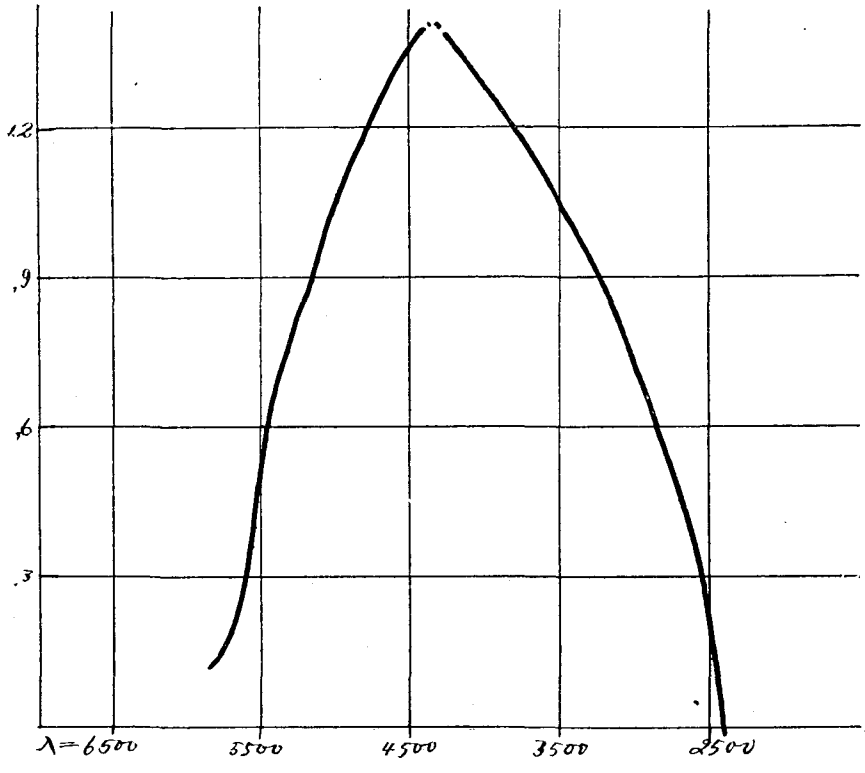
TABLE 18.

Concentration of scatole sol. and reagents	Wave-length of a boundary line of the absorption band (Hydrogen lamp)
Hydrogen lamp only	2250
Scatol 2·5% only	2450
5cc scatol 2·5%+1cc p-dimethyl+2cc HCL ...	2550-2495
„ 0·5%— „ „	3180-5050
„ 1·0%— „ „	3570-4585
„ 2·0%— „ „	4300-4370

Here we trace the following curve from the above results.

1) EHRLICH:—Deuts. med. Wochenschr. (1901).

Fig. 16.



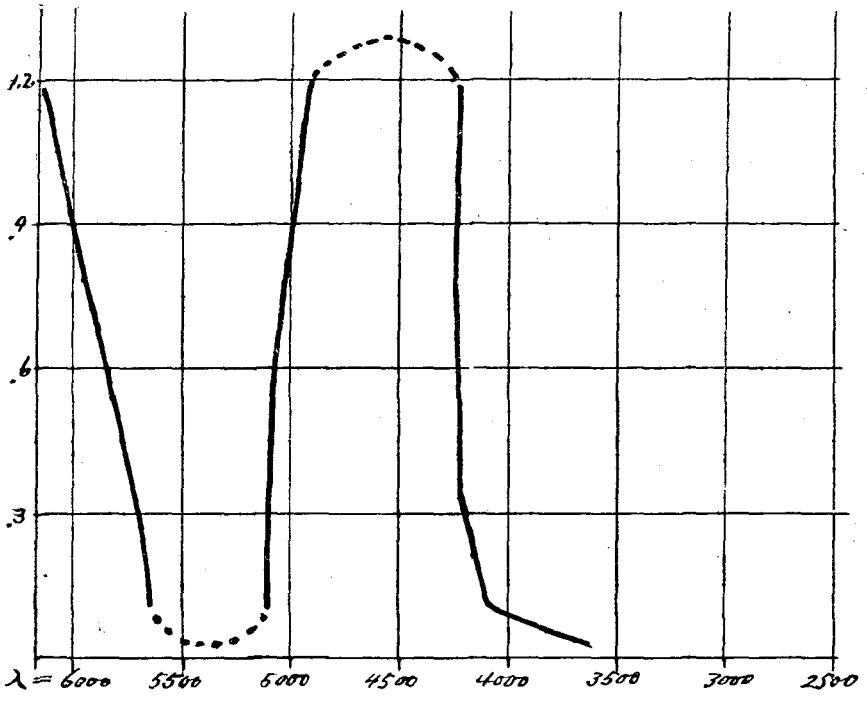
The result shows two absorptionbands between 6500–4500$\mu\mu$ and 3000–4000$\mu\mu$. The same reaction was examined using an electric lamp and we obtained the following results.

TABLE 19.

Concentration of scatol solution and reagents	Wave-length of a boundary line of the absorption band (Electric lamp)
Water only	3310
5cc H_2O+1cc 2% dimethylaminobenzaldehyd+2cc H_2O	3495
5cc 0·01% scatol- " "	3585
" 0·025% " " "	3625
" 0·05% " " "	3675
" 0·075% " " "	3710
" 0·10% " " "	4100–5120–5625
" 0·25% " " "	4175–5120–5755
" 0·50% " " "	4175–5000–6000
" 1·% " " "	4175–4900–6150

Here we trace the following curve from the above results.

Fig. 17. (See Phot. J.)



The above results showed two absorption bands between 6500–4500$\mu\mu$ and 3500–4000$\mu\mu$. The position of the curves remaining the same as in the above experiment.

I. *Comparison of the intensity of color-reactions.*

The comparison of the intensity of the different color reactions of the protein decomposition products was carried on by means of the above experimental results. The color intensity was tabulated according to the maxmum dilution of the solution, this showed a definite absorption band (layers of 10–11 mm thickness).

TABLE 20.

Name of sample	Reactions	
Creatin	Weyl's reaction .....0·06%	Jaffe's reaction .....0·125%
Tryptophan	Glyoxylic acid reaction.....0·10%	Adamkiewitz's reaction ...0·025%
Tyrosin	Wurster's reaction .....0·05–%	Diazo-reaction .....0·01–0·05%
Histidin	Diazo-reaction .....0·0002%	
Uric acid	Phosphotungstic acid react. 0·5%	K-bichromate react.....0·25–0·5%
Scatol	P-dimethylbenzaldehyd react. ..... 0·025–0·05%	

Part III. Some color reactions of glucosides and alkaloids.

J. **Arbutin.**

16. Nitric acid reaction:—Arbutin solution shows a green color reaction with fuming nitric acid[1]. We examined this reaction with an ultraspectroscope and obtaining the following results.

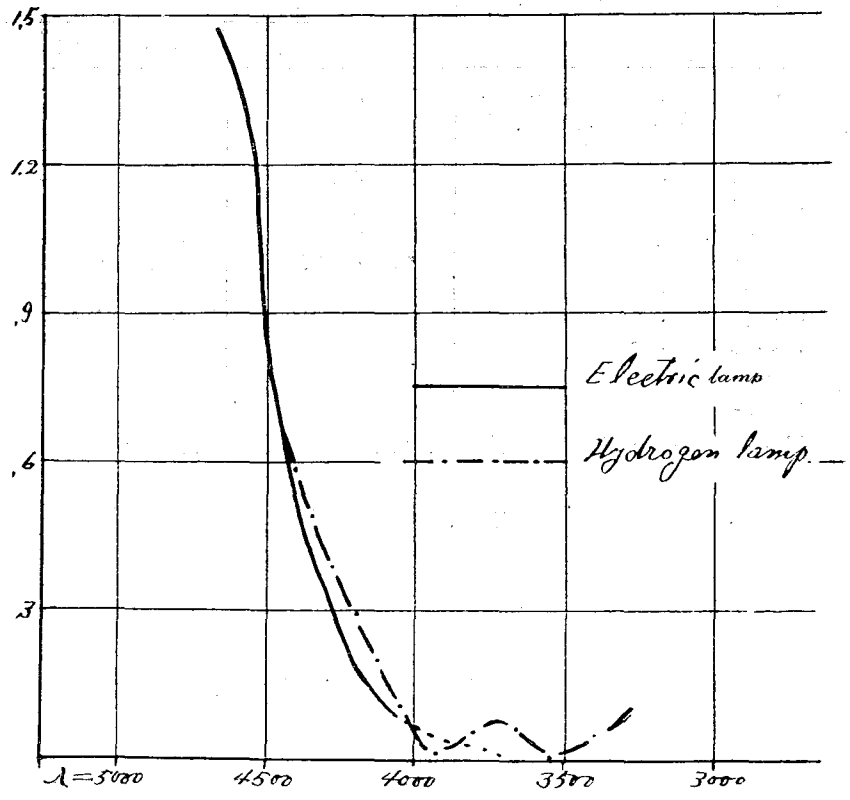
TABLE 21.

Concentration of arbutin sol. and Reagents	Wave-length of a boundary line of the absorption band	
	Electric lamp	Hydrogen lamp
0·1% arbutin only	3260	3245
8cc H_2O+2cc HNO_3...	3435	3275
8cc 0·001% arbutin+2cc HNO_3 ...	——	3320–3665–3925
„ 0·0025 „ „	4145	4100
„ 0·005 „ „	4175	——
„ 0·0075 „ „	4275	4220
„ 0·010 „ „	4375	——
„ 0·015 „ „	4475	4475
„ 0·025 „ „	4525	——
„ 0·050 „ „	4525	——
„ 0·075 „ „	4575	——
„ 0·10 „ „	4650	——

Here we trace the following curve from the above results.

1) REICHARD:—Pharmaz. Centrabl., 47, 555 (1906).

Fig. 18. (See phot. K.)



In the case of the electric lamp, there is only one curve in the visible portion between 5000–4000$\mu\mu$, and in the case of the hydrogen-lamp there are two curves in both the visible and invisible portions between 5000–3600$\mu\mu$ and 3600–3200$\mu\mu$.

K. **Salicin.**

17. Sulphuric acid reaction:—Salicin solution shows a red color reaction with sulphuric acid[1]. We examined this reaction with an ultraspectroscope and obtained the following results.

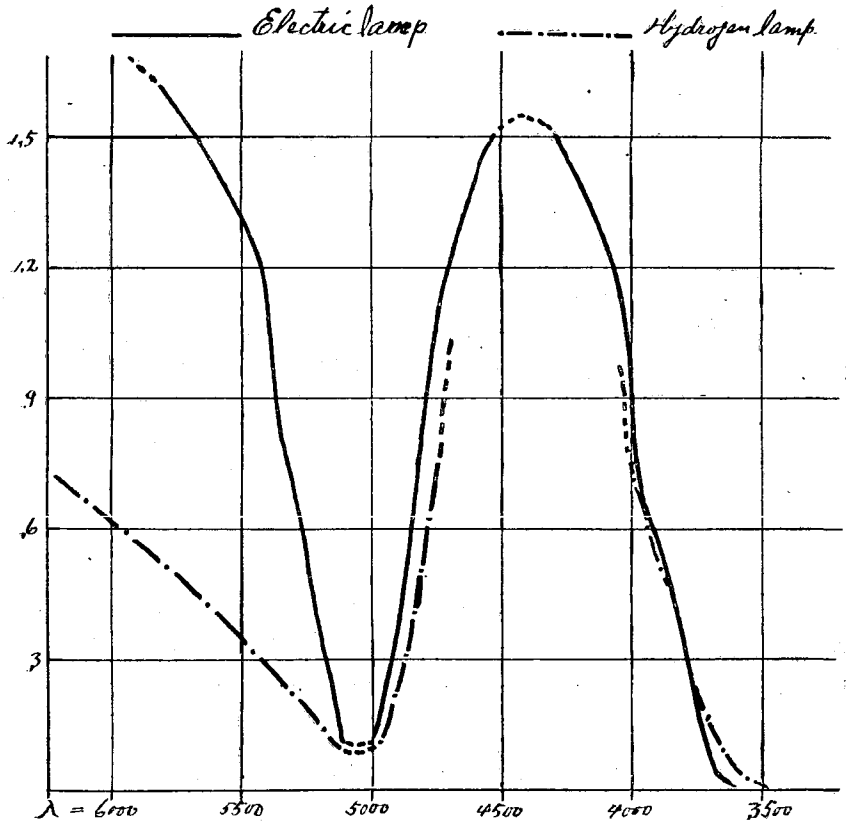
[1] PIRIA:—Journ. f. prakt. Chemie, 17, 242 (1839).

TABLE 22.

Concentration of salicin solution and reagents	Wave-length of a boundary line of the absorption band	
	Hydrogen lamp	Electric lamp
5cc H_2O+5cc H_2SO_4	3390	3320
5cc 0.001% salicin sol.+5cc H_2SO_4	3380	3570
„ 0.002 „ „	3420	3640
„ 0.004 „ „	5160–4950–3700	5150–4970–3720
„ 0.005 „ „	——	5150–4970–3785
„ 0.008 „ „	——	5201–4870–3835
„ 0.016 „ „	6420–4710–3980	5315–4820–3935
„ 0.035 „ „	——	5425–4795–4015
„ 0.050 „ „	——	5425–4675–4050
„ 0.075 „ „	——	5550–4600–4195
„ 0.100 „ „	——	5750–4525–4300
„ 0.125 „ „	——	5800

Here we trace the following curves from the above results.

Fig. 19. (See phot. L.)



These results showed two absorption bands between 4500–6000$\mu\mu$ and 3300–4200$\mu\mu$.

L. **Gallotannic acid.**

18. Potassium cyanide reaction:—Gallotannic acid solution shows a reddish brown color reaction with potassium cyanide.[1] We examined this reaction with, an ultraspectroscope and obtained the following results.

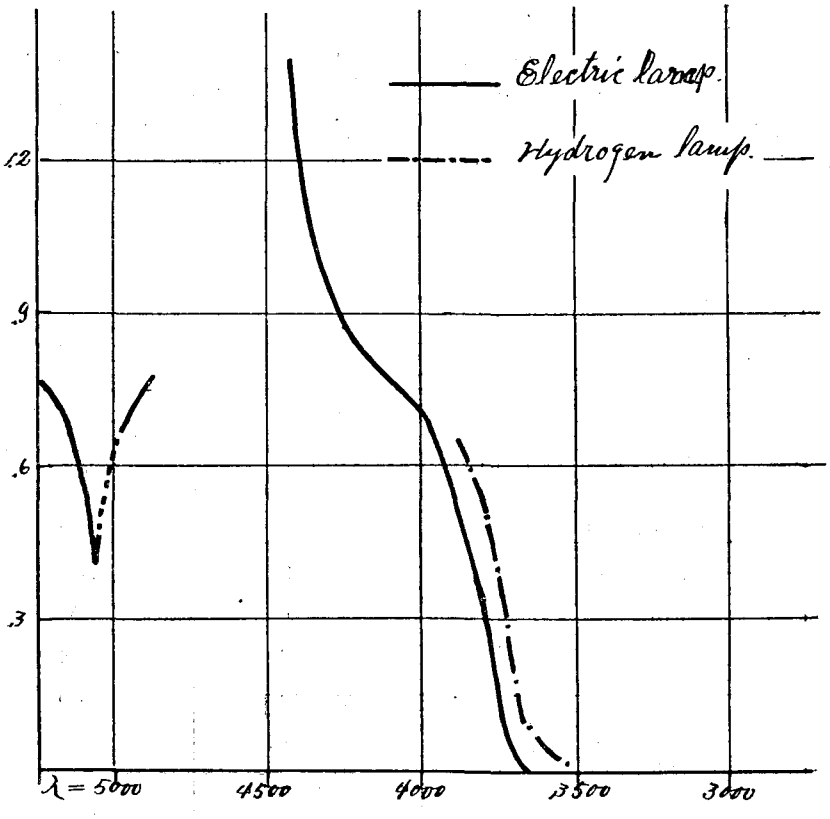
TABLE 23.

Concentration of gallotannic acid solution and reagents	Wave-length of a boundary line of the absorption band	
	Electric lamp	Hydrogen lamp
0.1% gallotannic acid solution only	3245	2260
5cc 0.001% „ +1 drop KCN (1%)	3395	3525
5cc 0.002 „ „	3770	3665
„ 0.005 „ „	5075–3845	3725
„ 0.0075 „ „	5100–3920	3880
„ 0.010 „ „	5150–4950–3980	——
„ 0.0125 „ „	4115	——
„ 0.015 „ „	5275–4870–4200	——
„ 0.025 „ „	4295	——
„ 0.050 „ „	4355	——
„ 0.100 „ „	4378	——

Here we trace the following curves from the above results.

1) Biochem. Jour., 2, 96 (1907).

Fig. 20. (See phot. MA.)



These results showed two absorption bands between 5500–4700$\mu\mu$ and 4200–3200$\mu\mu$.

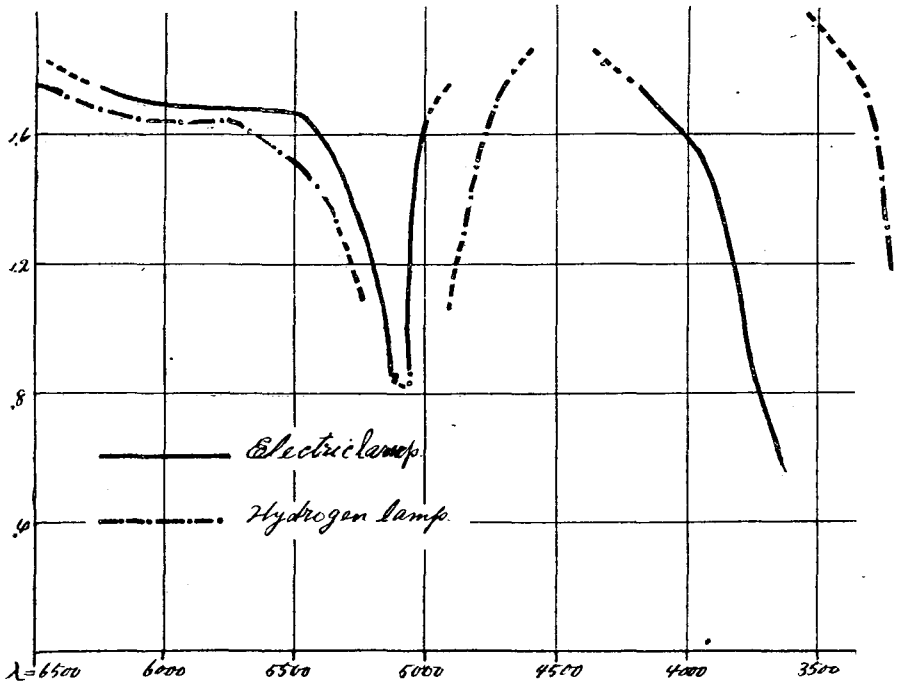
19. Ferric chloride reaction:—Gallotannic acid solution shows a blue color reaction with ferric chloride (l. c.). We examined as above.

TABLE 24.

Concentration of gallotannic acid sol.	Reagents	Wave-length of a boundary line of the absorption band	
		Electric lamp	Hydrogen lamp
Water only		3275	2250
5cc H_2O	+ 1cc 1·9% $FeCL_4$	3525	2325
„ 0·002%	gallotannin + 1cc 1·% $FeCL_3$	3655	——
„ 0·055	„ „	5150–5050–3745	——
„ 0·075	„ „	3800	——
„ 0·10	„ „	5250–5050–3875	——
„ 0·125	„ „	3925	5350–4825–2920
„ 0·15	„ „	5450–5000–4100	5725–4750–3215
„ 0·175	„ „	6250–4155	6450–4675–3270

Here we trace the following curves from the above results.

Fig. 21. (See phot. M.B)



These results showed two absorption bands between 3500–4300$\mu\mu$ and 5000–6250$\mu\mu$.

M. **Salicylic acid.**

20. Ferric chloride reaction.—Salicylic acid solution shows a purple red color with ferric chloride solution.[1] We examined this reaction with an ultraspectroscope and obtained the following results.

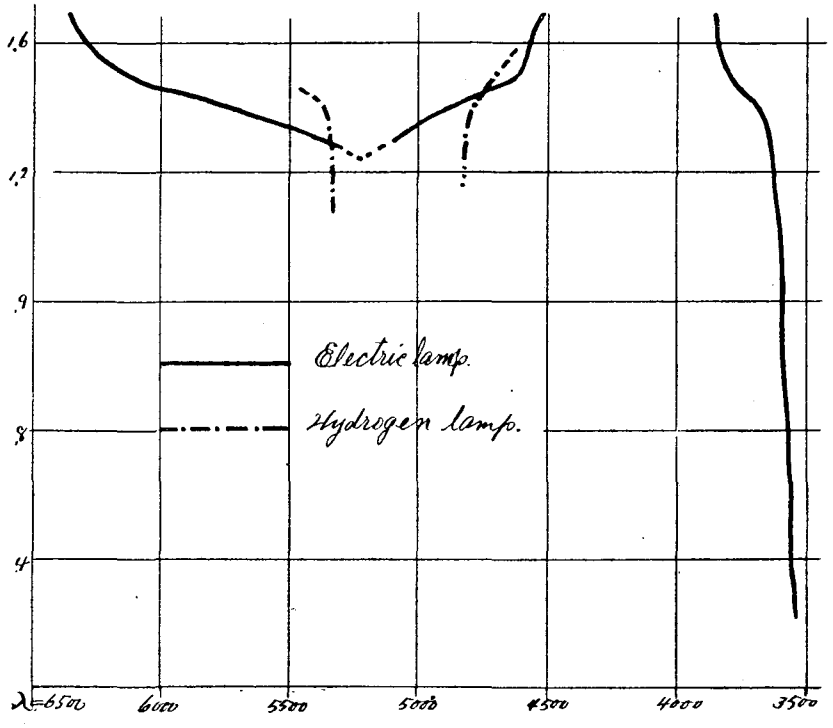
TABLE 25.

Concentration of salicylic acid solution and reagents	Wave-length of a boundary line of the absorption band	
	Electric lamp	Hydrogen lamp
8cc H_2O+1 drop 1% $FeCL_3$ solution	3345	—
8cc 0.1% salicylic acid solution-only	3475	—
8cc 0.001% salicyl. sol.+1 drop $FeCL_3$ sol.	3525	—
8cc 0.002% ,, ,,	3535	—
8cc 9.005% ,, ,,	3570	—
8cc 0.0075% ,, ,,	3590	—
8cc 0.010% ,, ,,	3600	—
8cc 0.025% ,, ,,	3625	—
8cc 0.05% ,, ,,	5325-5075-3645	5350-4800
8cc 0.075% ,, ,,	5750-4870-3675	5350-4800
8cc 0.010% ,, ,,	4650-3780	4700
8cc 0.125% ,, ,,	6300-4575-3825	—
8cc 0.150% ,, ,,	6350-4510-3840	—

Here we trace the following curves from the above results.

1) AUTENLIETH:—Der Auffindung d. Gifte, Aufl. 4, 67, 1909.

Fig. 22. (See phot. N.)



These results showed two absorption bands between 3300–3900$\mu\mu$ and 4500–6500$\mu\mu$.

N. **Antipyrin.**

21. Nitric acid reaction:—Antipyrin solution shows a red color reaction with fuming nitric acid.[1] We examined this reaction with an ultraspectroscope and obtained the following results.

---

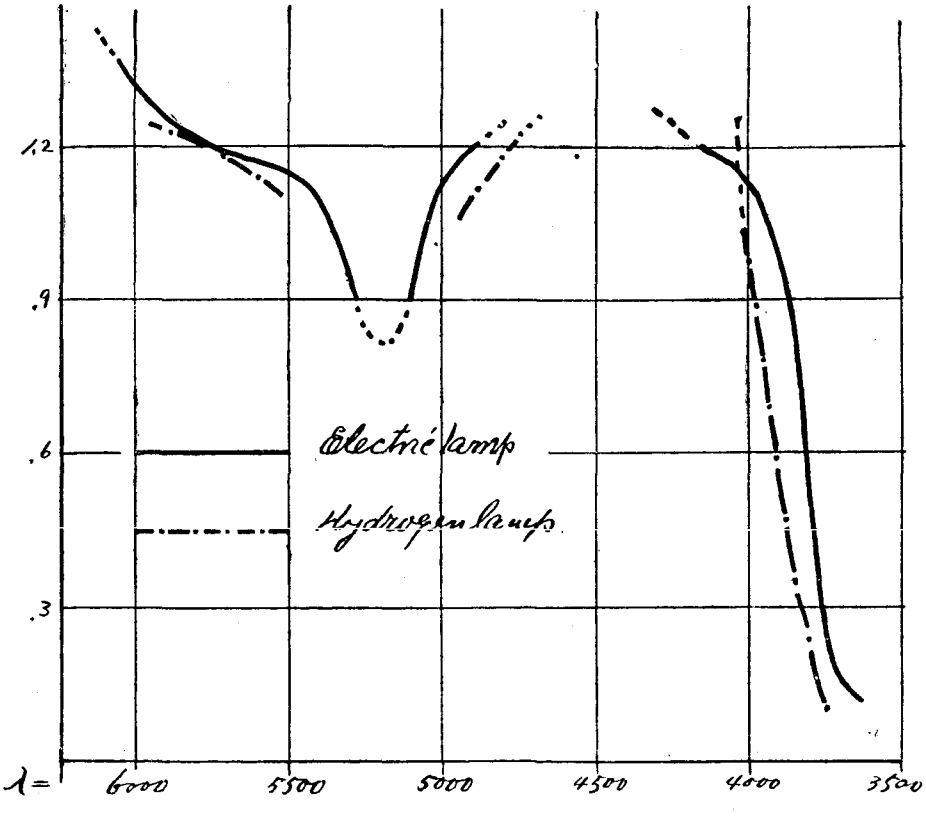
1) Zs. physiol. Chem., 80, 17 (1912).

TABLE 26.

Concentration of antipyrine solution and reagents	Wave-length of a boundary line of the absorption band	
	Electric lamp	Hydrogen lamp
0·1% antipyrin solution only...	3315	——
5cc 0·001% antipyrin 2cc HNO_3	3375	——
5cc 0·002% ,,	3460	——
5cc 0·004% ,,	3535	3535
5cc 0·0075% ,,	3635	3760
5cc 0·010% ,,	3730	——
5cc 0·025% ,,	3800	——
5cc 0·050% ,,	5300–5100–3865	——
5cc 0·075% ,,	5350–5050–3935	4000
5cc 0·090% ,, ,,	5410–5025–3985	——
5cc 0·100% ,, ,,	5725–4895–4145	5700–4770–4000
5cc 0·125% ,, ,,	5959	——
5cc 0·150% ,, ,,	6050	——

Here we trace the following curves from the above results.

Fig. 23. (See phot. O.A)



These results showed the absorption bands between 3300–4000$\mu\mu$ and 4500–6000$\mu\mu$.

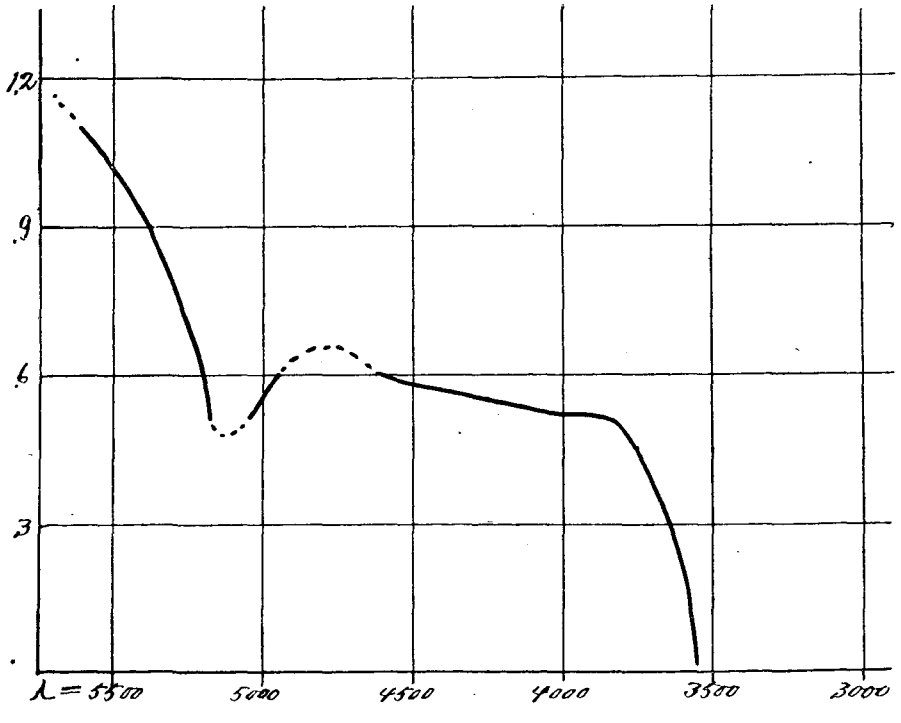
22. Ferric chloride solution :—Antipyrin solution shows a red color reaction with ferric chloride (l.c.). We examined this reaction with an ultraspectroscope and obtained the following results.

TABLE 27.

Concentration of antipyrine solution and reagents	Wave-length of a boundary line of the absorption band (Electric lamp)
0·1% antipyrine solution only	3285
8cc 0·001% antipyrine sol.+1 drop $FeCL_3$ sol.	3580
„ 0·002% „ „	3600
„ 0·004% „ „	3635
„ 0·008% „ „	3665
„ 0·010% „ „	3735
„ 0·0125% „ „	3175–5050–3795
„ 0·015% „ „	5200–4950–4625
„ 0·030% „ „	5375
„ 0·050% „ „	5600

Here we trace the following curve from the above results.

Fig. 24. (See phot. O.B)



These results showed the absorption bands between 3300–4500$\mu\mu$ and 4500–5500$\mu\mu$.

O. **Quinin.**

23. Bromine water reaction:—Quinin hydrochloride solution shows a green color reaction with bromine water and ammonia.[1] We examined this reaction with an ultraspectroscope and obtained the following results.

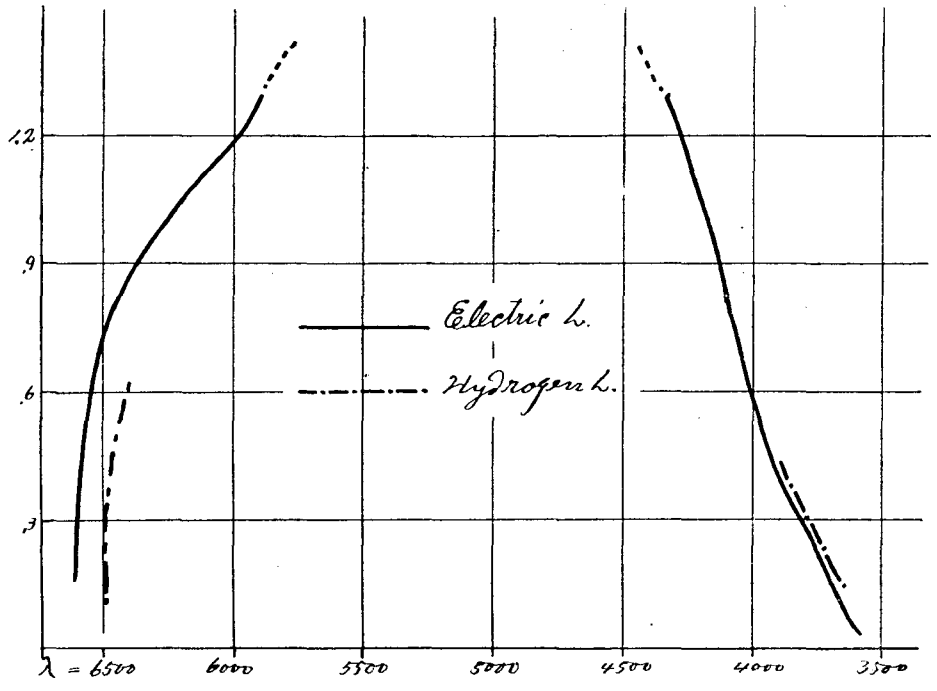
TABLE 28.

Concentration of quinin solution and reagents	Wave-length of a boundary line of the absorption band	
	Electric lamp	Hydrogen lamp
0.1% quinin HCL solution only	3245	2240
8cc H_2O + 1cc brom-water + 1cc NH_4OH	3315	2340
8cc 0.0025% quinin + 1cc brom-water + 1cc NH_4OH	3525	——
8cc 0.005% ,, ,, ,,	3575	——
8cc 0.0075% ,, ,, ,,	3795	5450–3780
8cc 0.010% quinin + 1cc brom-water + 1cc NH_4OH	6680–3885	——
8cc 0.015% ,, ,, ,,	6525–4045	——
8cc 0.025% ,, ,, ,,	6500–4075	——
8cc 0.050% ,, ,, ,,	6015–4275	——
8cc 0.075% ,, ,, ,,	5950–4275	——
8rc 0.010% ,, ,, ,,	5900–4315	——

Here we trace the following curves from the above results.

1) ABDERHALDEN:—Biochem. Arbeitmethoden, V., 1, 119 (1912).

Fig. 25 (See phot. P.)



These results showed two absorption bands between 3500–4300$\mu\mu$ and 6900–6600$\mu\mu$.

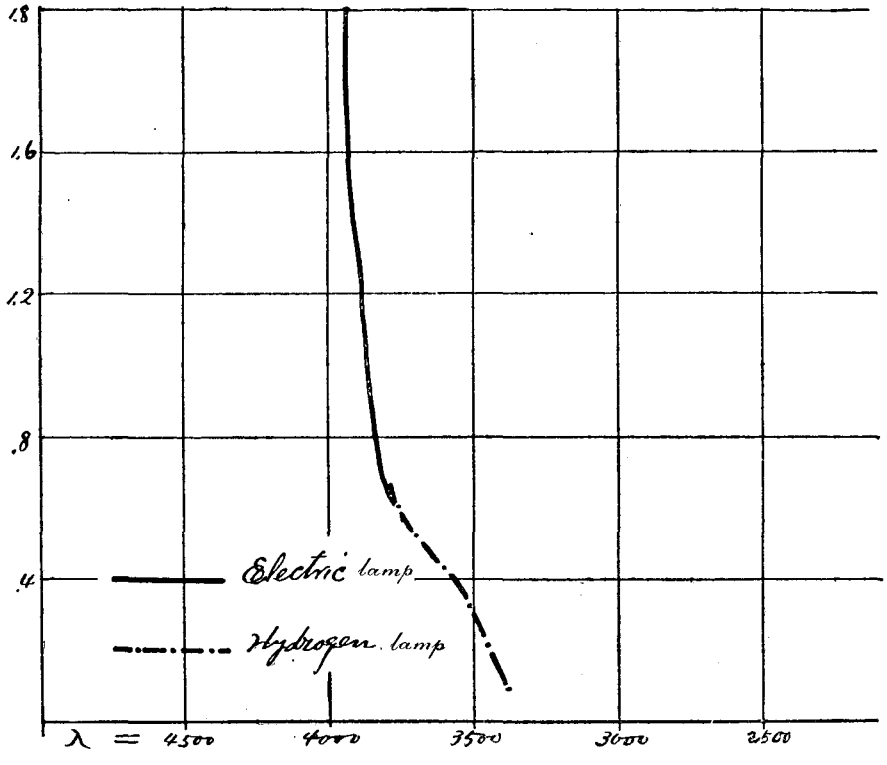
24. Sulphuric acid reaction :—Quinin hydrochloride solution shows a blue fluoresence with strong sulphuric acid (l.c.). We examined this reaction by the same treatment and obtained following results.

TABLE 29.

Concentration of quinin solution and reagents	Wave-length of a boundary line of the absorption band	
	Electric lamp	Hydrogen lamp
0·1% quinin HCL solution only	3265	3310
5cc H_2O+2cc H_2SO_4	3420	3320
5cc 0·001% quinin HCL sol. +2cc H_2SO_4 ...	——	3465
,, 0·00125% ,, ,,	——	3580
,, 0·00150% ,, ,,	——	3615
,, 0·00175% ,, ,,	——	3625
,, 0·0020% ,, ,,	3755	3765
,, 0·0025% ,, ,,	3840	——
,, 0·005% ,, ,,	3875	——
,, 0·0075% ,, ,,	3895	——
,, 0·010% ,, ,,	3895	——
,, 0·025% ,, ,,	3925	——
,, 0·050% ,, ,,	3525	——

Here we trace the following curves from the above results.

Fig. 26.



This result showed one absorption band between 3500–3925$\mu\mu$.

P. **Codein.**

23. MARQUIS' reaction :—Codein phosphate solution shows a bluish violet color with MARQUIS' reagent.[1] We examined this reaction with an ultrospectroscope and obtained the following results.

---

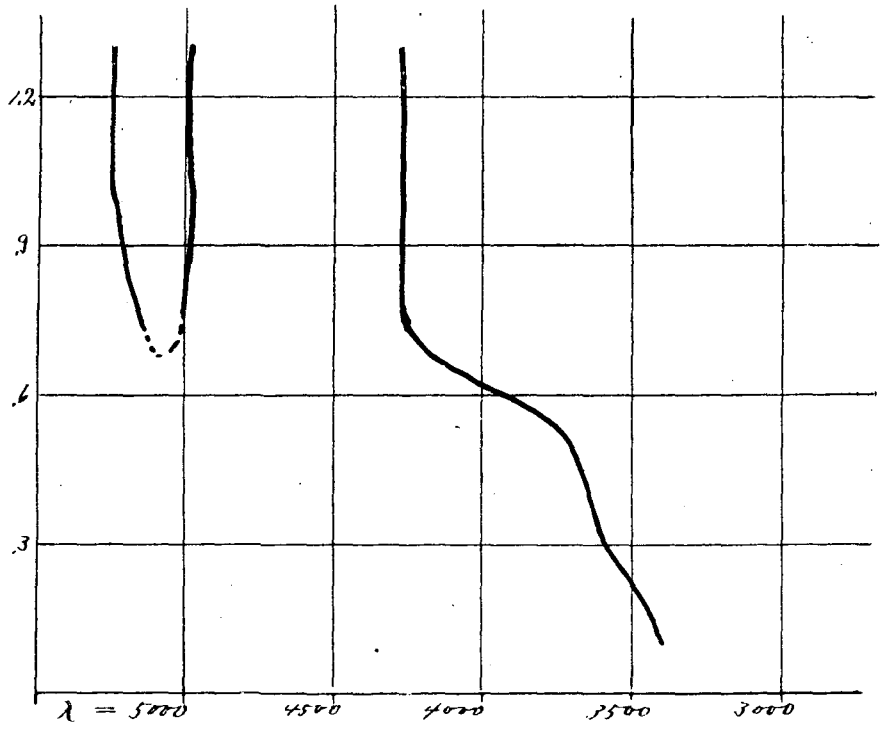
1) ABDERHALDEN :—Biochem. Arbeitmethode, VI., 119 (1912).

TABLE 30.

Concentration of codein solution and reagents	Wave-length of boundary line of the absorption band (Electric lamp)
0·1% codein phosphate solution only	3265
5cc H_2O+1 drop formalin water+H_2SO_4	3393
5cc 0·001% codein+ " "	3425
" 0·0025% " "	3465
" 0·005% " "	3475
" 0·0075% " "	3485
" 0·010% " "	3535
" 0·0125% " "	3575
" 0·015% " "	3655
" 0·025% " "	5195–5000–4225
" 0·050% " "	5250–4990–4275
" 0·100% " "	5250–4990–4265

Here we trace the following curve from the above results.

Fig. 27. (See phot. Q.A)



These results showed two absorption bands between 5000–5200$\mu\mu$ and 3300–4300$\mu\mu$.

25. FROEDES reaction :—Codein phosphate solution shows a reddish brown color with Froedes reagent, *i.e.* molybdic acid and sulphuric acid.[1] We examined this reaction with an ultraspectroscope and obtained the following results.

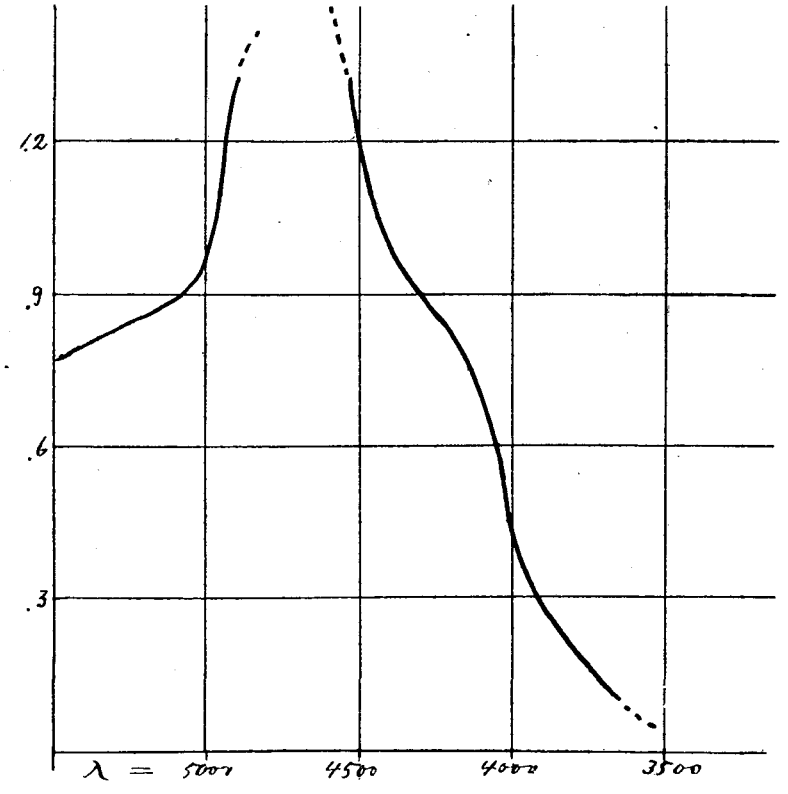
TABLE 31.

Concentration of codein solution and reagents	Wave-length of boundary line of the absorption band (Electric lamp)
0·1% codein phosphate	3345
5cc 0·001% codein phos.+1drop molybdic acid +3cc H_2SO_4	3545
5cc 0·002% ,, ,, ,,	3670
,, 0·004% ,, ,, ,,	3745
,, 0·008% ,, ,, ,,	3765
,, 0·016% ,, ,, ,,	5750–4035
,, 0·035% ,, ,, ,,	5000–4200
,, 0·050% ,, ,, ,,	4955–4365
,, 0·075% ,, ,, ,,	4902–4475
,, 0·0125% ,, ,, ,,	4501

Here we trace the following curve from the above results.

1) ABDERHALDEN: (l.c.)

Fig. 28. (See phot. Q.B)



These results showed two absorption bands between 6600–4800$\mu\mu$ and 3500–4500$\mu\mu$.

Q. **Strychnin.**

26. K-Bichromate reaction:—Strychnin solution showed a red color reaction with Potassium bichromate and sulphuric acid.[1] We examined this reaction with an ultraspectroscope and obtained the following results.

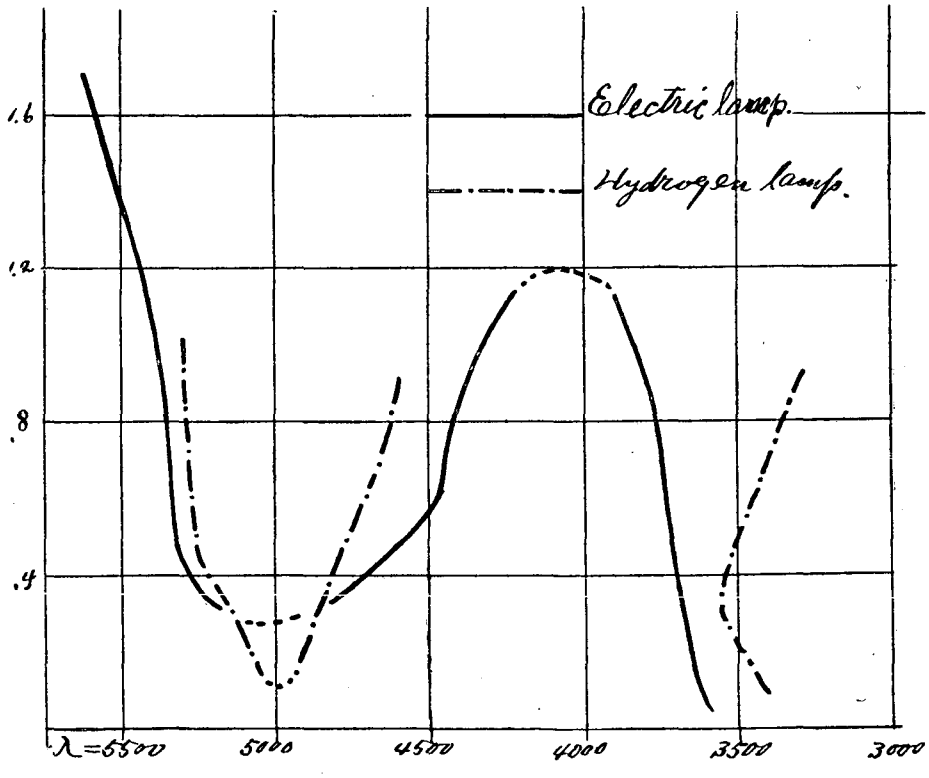
1) ABDERHALDEN: (l.c.)

TABLE 32.

Concentration of strychnin solution and reagents	Wave-length of a boundary line of the absorption band	
	Electric lamp	Hydrogen lamp
5cc H_2O+5cc H_2SO_4	——	3115
5cc 0·1% strychnin solution only...	3245	3125
5cc 0·001% „ +5cc H_2SO_4	3640	3125
„ 0·0025% „ — „	3675	5100–4935–3510
„ 0·005% „ — „	3690	——
„ 0·0075% „ — „	5190–4990–3720	5175–4825–3715
„ 0·010% „ „	5335–4475–3755	5250–4800–3525
„ 0·025% „ „	5355–4435–3775	5275–4625–3320
„ 0·050% „ „	5400–4225–3865	——
„ 0·075% „ „	5485	——
„ 0·100% „ „	5500	——
„ 0·125% „ „	5550	——
„ 0·150% „ „	5595	——

Here we trace the following curves from the above results.

Fig. 29. (See phot. R.)



These results showed two absorption bands between 5500–4500$\mu\mu$ and 3150–4000$\mu\mu$.

R. **Nicotin.**

27. Formalin and nitric acid reaction :—Nicotin solution shows a red color reaction with formalin and nitric acid.[1] We examined this reaction with an ultraspectroscope and obtained following results.

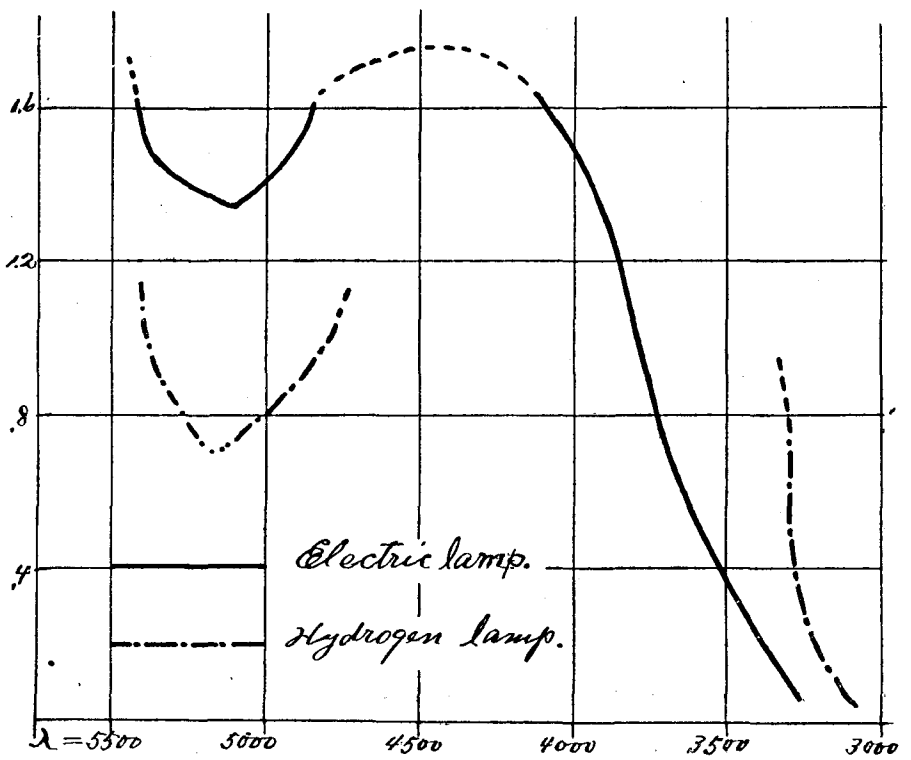
TABLE 33.

Concentration of nicotin solution and reagents	Wave-length of boundary line of the absorption band	
	Electric lamp	Hydrogen lamp
5cc H_2O+1 drop formalin+2cc HNO_3	3325	2785
5cc 0.00025% nicotin sol.+1 drop formalin +2cc HNO_3	——	3265
„ 0.0005% „ „ „	——	3265
„ 0.00075% „ „ „	——	3285
„ 0.001% „ „ „	3480	——
„ 0.00125% „ „ „	3542	——
5cc 0.00150% „ „ „	3625	——
„ 0.00175% „ „ „	3685	3285
„ 0.0020% „ „ „	3750	5225-5100-3285
„ 0.0025% „ „ „	3795	5302-4925-3285
„ 0.005% „ „ „	3823	——
„ 0.0075% „ „ „	5125-3905	——
„ 0.010% „ „ „	5350-4950-3925	——
„ 0.0125% „ „ „	5400-4875-4035	——
„ 0.0150% „ „ „	5400-4825-4095	——

Here we trace the following curves from above results.

1) ABDERHALDEN :—(l.c.)

Fig. 30.



These results showed two absorption bands between 3300–4000$\mu\mu$ and 5400–4750$\mu\mu$.

S. **Morphin.**

28. MARQUIS' reaction:—Morphin solution shows a purple red color reaction with MARQUIS' reagent.[1] We examined this reaction with an ultraspectroscope and obtained the following results.

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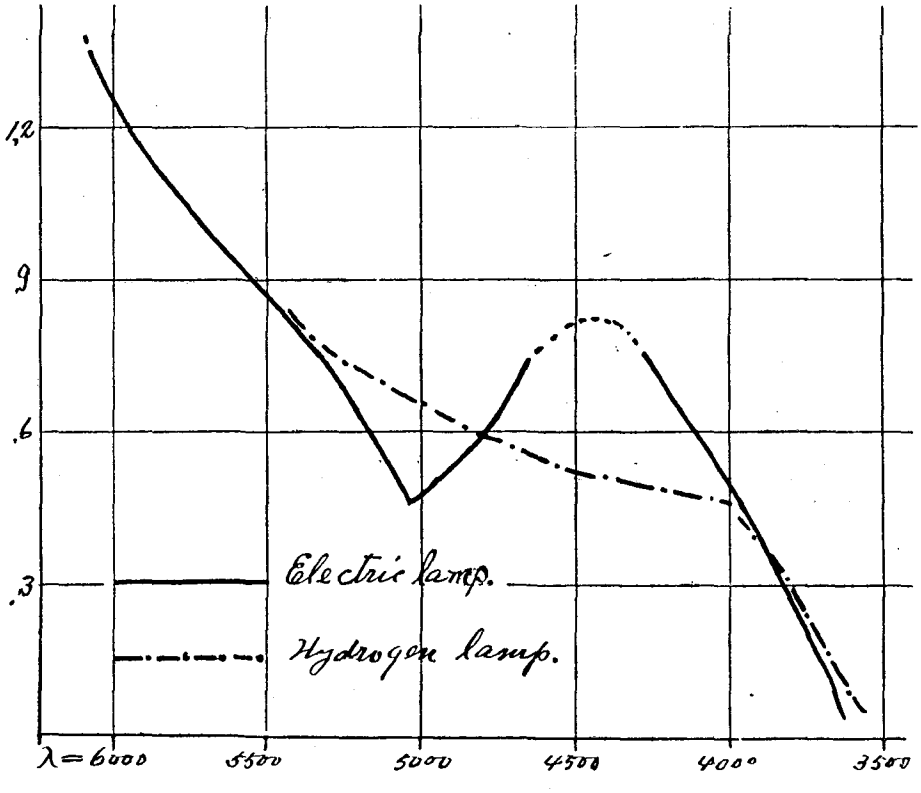
1) Zeits. physiol. Chem., 71, 273 (1911).

TABLE 34.

Concentration of morphin solution and reagents	Wave-length of a boundary line of the absorption band	
	Electric lamp	Hydrogen lamp
5cc 0.001% morphin sol. + 1 drop marquis reagent	3310	3415
5cc 0.002% ,, ,,	3555	—
,, 0.004% ,, ,,	3650	3620
,, 0.0075% ,, ,,	3825	3830
,, 0.0125% ,, ,,	3920–5025	3960
,, 0.025% ,, ,,	4620–4275–5325	5325
,, 0.050% ,, ,,	5725	—
,, 0.10% ,, ,,	6075	—

Here we trace the following curves from the above results.

Fig. 31.



These results showed two absorption bands between 3500–4000$\mu\mu$ and 4500–5500$\mu\mu$.

7. *Comparison of the intensity of color reactions.*

Comparing the different color reactions of the glucosides and alkaloids with the above experimented results, their intensity was tabulated by the minimum concentration of the solution, this showed the definite absorption band.

TABLE 35.

Name of sample	Reactions	
Arbutin	Nitric acid reaction..... 0·001–0·002%	
Salicin	Sulphric acid reaction ...0·002–0·004%	
Gallotannic acid	Potassium cyanic react. ...0·005%	Ferric chloride reaction.....0·04%
Salicylic acid	Ferric chloride react. .....0·045%	
Antipyrin	Nitric acid reaction.....0·045%	Ferric chloride reaction...0·0125%
Quinin	Bromin water reaction ...0·0075%	Sulphric acid reaction .....0·01%
Codein	Marquis' reaction .....0·02%	Froedes' reaction .....0·010%
Strychnin	Potassium bichromate reaction ...0·0025%	
Nicotin	Nitric acid reaction .... 0·002%	
Morphin	Marquis' reaction .....0·0125%	

The color reaction was examined with a quartz cell of 10 mm in thickness.

SUMMARY.

The results of the above experiments are summarized in the following table, comparing the position, the rate of growth and the intensity of the absorption bands of different color-reactions. We compared the position of the absorption bands with the wave-length which was absorbed at minimum concentration. We compared the position of the boundary lines of the absorption bands in the shorter and longer wave-lengths. The rate of growth of the absorption bands was compared with the form of their curves which are discribed in the table as sharp or dull. The intensity of the reaction was compared with the minimum concentration of the solution which showed a definite absorption band on the photographic plate.

TABLE 36.

Name of sample	Reaction	Position of the absorption band (in min. con.)	Position of the absorption band (boundary line)	Rate of growth	Minimum concentration
		Part I. Hexose.			
Glucose	Neuman' R.	5070	betw. 4900–5350	very sharp	1/8%
		No	„ 3500–4050		
Fructose	„	No	„ 3500–6100	grows gradually	
Mannose	„	5150	„ 4750–5350	sharp	1/4%
		No	„ 3500–4250	dull	
Galactose	„	5050	„ 4500–6000	dull	1/4%
		No	„ 3500–4400		
Glucose	Diazobenzene sul. R.	5400	„ 5750–5000	dull	
		No	„ 3500–4750		
Fructose	„	5300	„ 4900–5750	dull	
		No	„ 3500–4500		
Mannose	„	5300	„ 4500–5750	dull	
		No	„ 3500–4400		
		Part II. Pentose.			
Xylose	Phloroglucin R.	No	betw. 6000–5000		0·01%
		2650	„ 2600–3200		
Arabinose	„	No	„ 6000–5000		0·025%
		2650	„ 2600–3200		

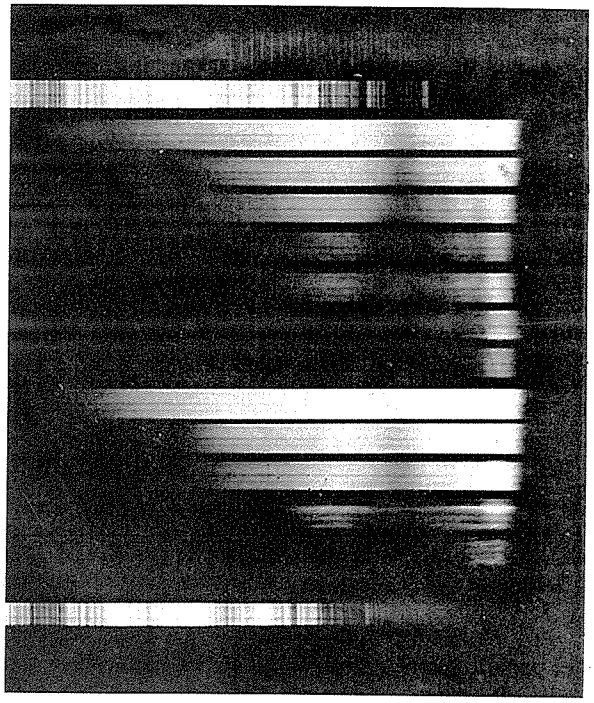
Name of sample	Reaction	Position of the absorption band (in. min. con.)	Position of the absorption band (boundary line)	Rate of growth	Minimum concentration
Xylose	Neuman's R.	5900	betw. 5350–6250	dull	0.15%
		5100	„ 5350–4900		
		No	„ 3750–4100		
Arabinose	„	5900	„ 5450–6250	dull	0.025%
		5100	„ 5450–4900		
		No	„ 3850–4200		
Furfurol	Anilin acetate R.	No	„ 3600–4300	grows gradually	
Part III. Protein decomposition products.					
Creatinin	Weyl's R.	4050	betw. 4000–3700	very dull	0.06%
		No	„ 2800–3500		
	Jaffe's R.	No	„ 7000–6700		0.125%
		No	„ 4800–4000		
Tryptophan	Glyoxylic A.R.	5700	„ 6250–5000	dull	0.10%
		4600	„ 4800–4000		
		No	„ 2500–2800		
	Adamkiewicz's R.	4700	„ 4000–5000	sharp	0.025%
		No	„ 3500–2500		
Tyrosin	Wurster's R.	5000	„ 5500–4800		0.05–0.10%
		No	„ 6000–6500		
		No	„ 3500–4500		0.01–0.05%
	Diazobenzene sul. R.	No	„ 3000–5500		
Histidin	„	5250	„ 5500–4800	sharp	0.0002%
		No	„ 2990–3500		
Uric acid	Phosphotungstic A.R.	No	„ 3000–3500		0.05%
	Pot-Bichromate R.	3400	„ 3200–3600		
		No	„ 3000–3200		0.25–0.5%
		No	„ 6000–6500		
Scatol	p-Dimethylbenzaldehyd R.	5400	„ 6500–4500	sharp	0.025–0.05%
		No	„ 3000–4000		
Part IV. Glucosides and alkaloids.					
Arbutin	Nitric acid R.	No	betw. 5000–3600	dull	0.001–0.002%
			„ 3600–3200		
Salicin	Sulphuric acid R.	5050	„ 6000–4500	sharp	0.002–0.004%
		No	„ 3300–4200		

Name of samples	Reaction	Position of the absorptionband (in. min. con.)	Position of the absorptionband (boundary line)	Rate of growth	Minimum concentration
GallotannicA.	Pot-Cyanide R.	5100	betw. 5500–4700	sharp	0·005%
		No	„ 4200–3200		
	Ferric chloride R.	5100	„ 5000–6250	very sharp	0·04%
		No	„ 3500–4300		
Salicylic acid	Ferric chloride R.	5200	„ 4500–6500	dull	0·045%
		No	„ 3300–3900		
Antipyrin	Nitric acid R.	5150	„ 6000–4500	sharp	0·0045%
		No	„ 3300–4000		
	Ferric chloride R.	5150	„ 5500–4500	sharp	0·0125%
		No	„ 3300–4500		
Quinin	Bromin water	No	„ 6900–6000		0·0075%
		No	„ 3500–4300		
	Sulphuric acid R.	No	„ 3500–3925		0·01%
Codein	Marquis' Reaction	4850	„ 5000–5200	very sharp	0·02%
		No	„ 3300–4500		
	Froedes' Reaction	No	„ 6690–4800		0·010%
		No	„ 3350–4500		
Strychnin	Pot.-Bichromate R.	5000	„ 5500–4500	dull	0·0025%
		No	„ 3150–4000		
Nicotin	Nitric acid R.	5200	„ 4750–5400	dull	0·002%
		No	„ 3300–4000		
Morphin	Marquis R.	5050	„ 5500–4500	dull	0·0125%
		No	„ 3500–4000		

The author wishes to express his hearty thanks to Prof. STIEGLITZ and Dr. WENDT in the Kent chemical laboratory, university of Chicago for the valuable suggestions they gave in carrying out this research.

Phot. B.

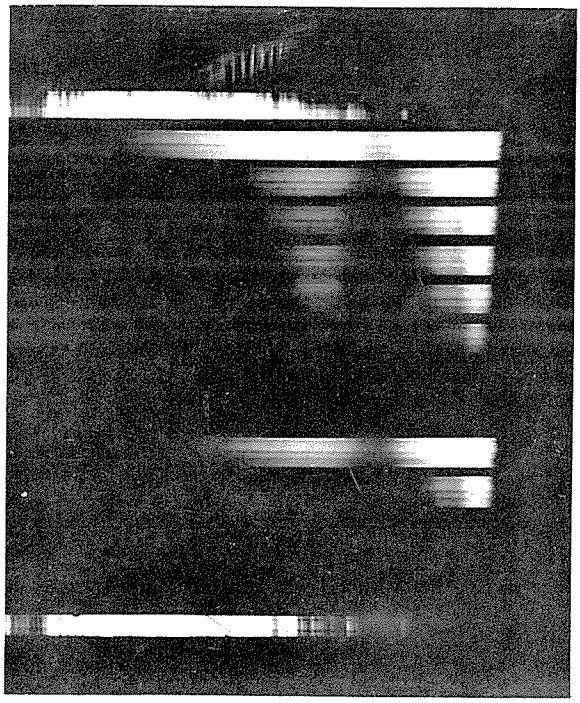
Mannose.



Galactose.

Phot. A.

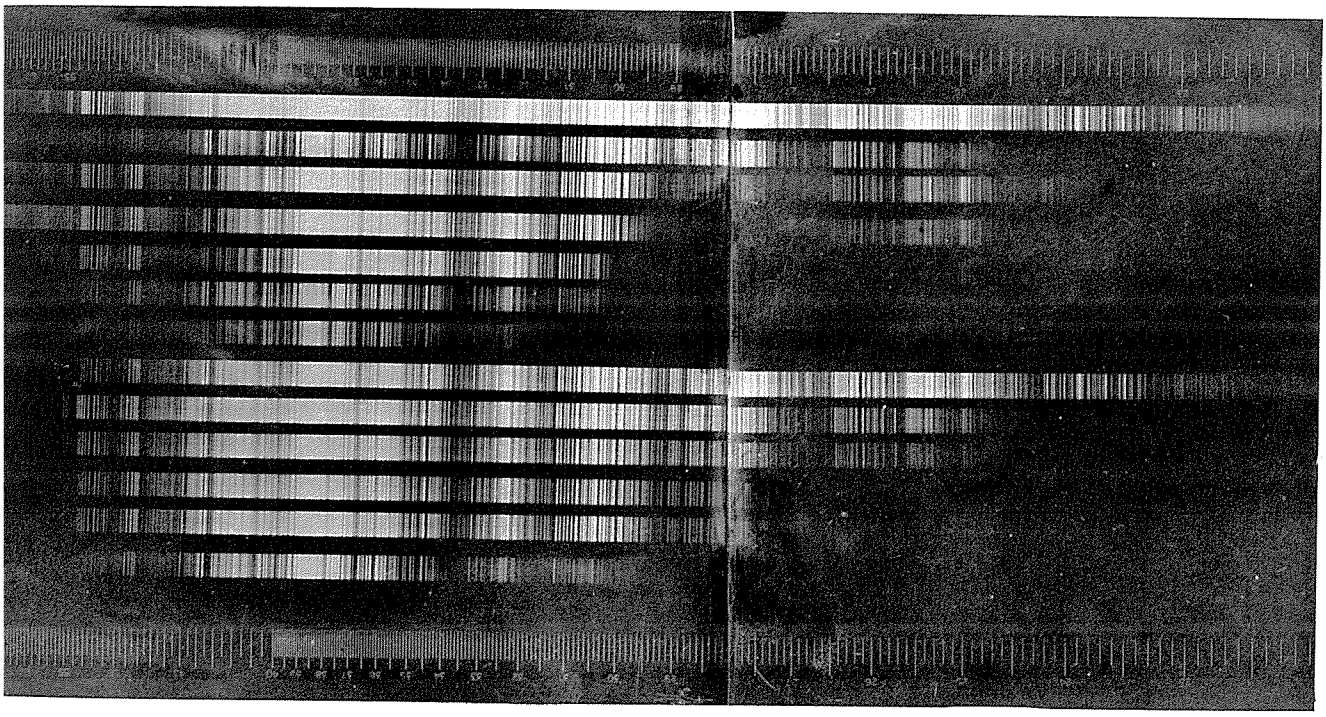
Glucose.



Fructose.

Phot. C_A.

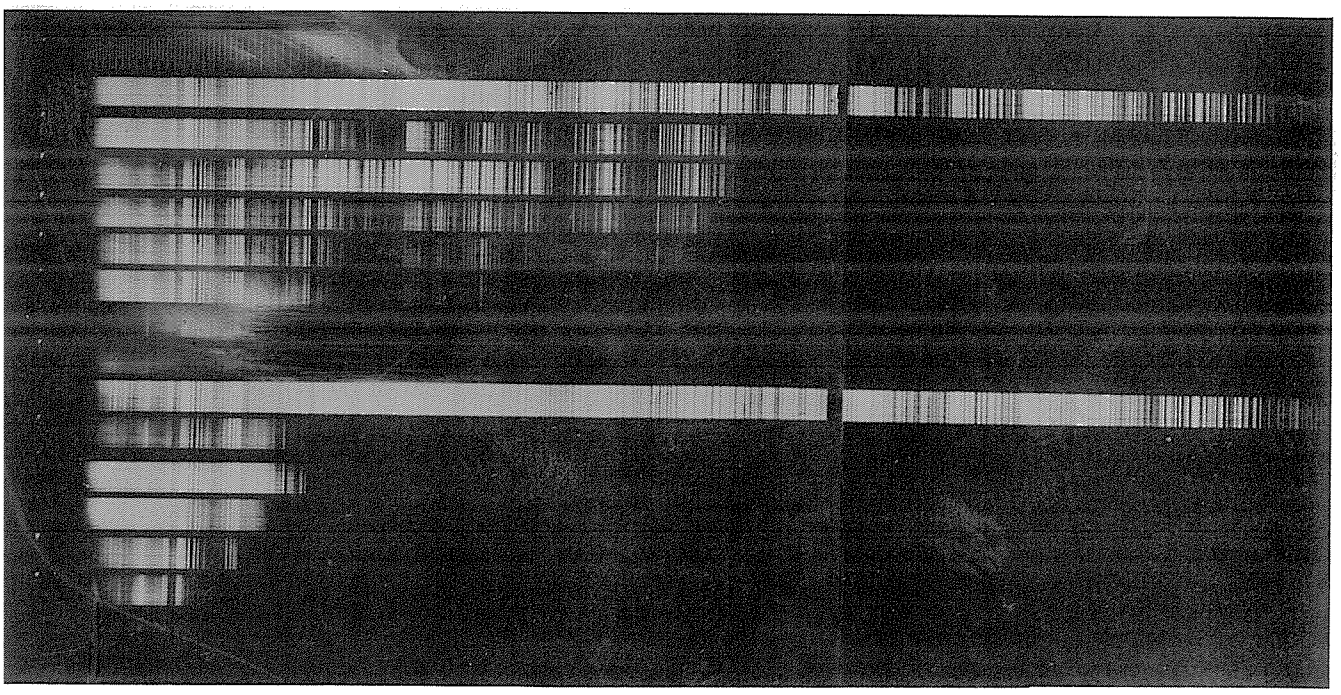
Xylose.



Arabinose.

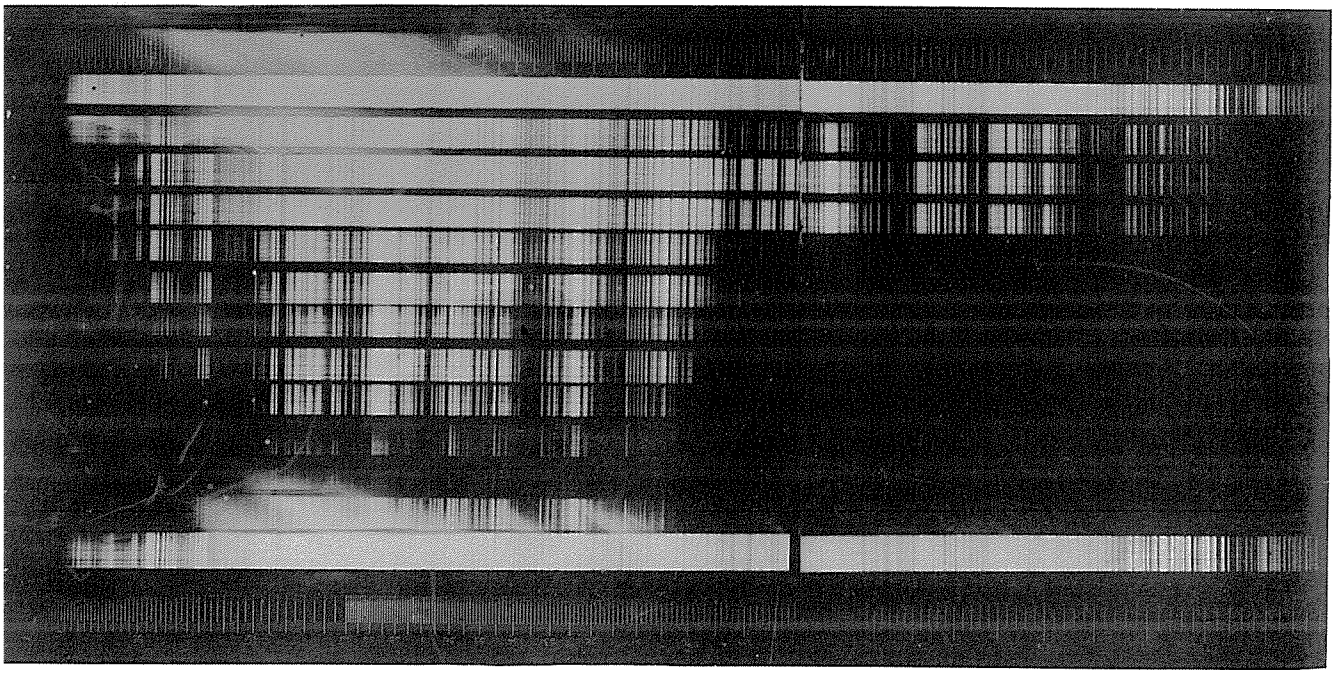
Phot. E.

Weyl's reaction.

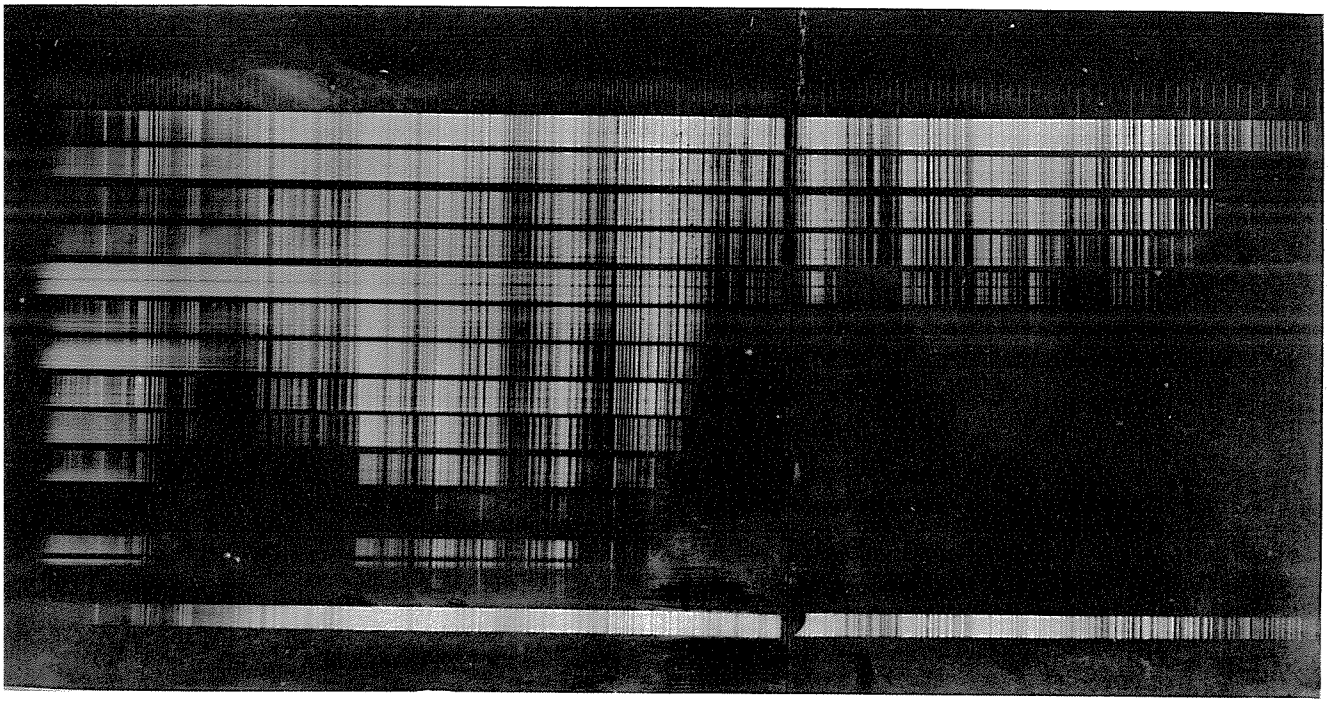


Jaffe's reaction.

Phot. F.



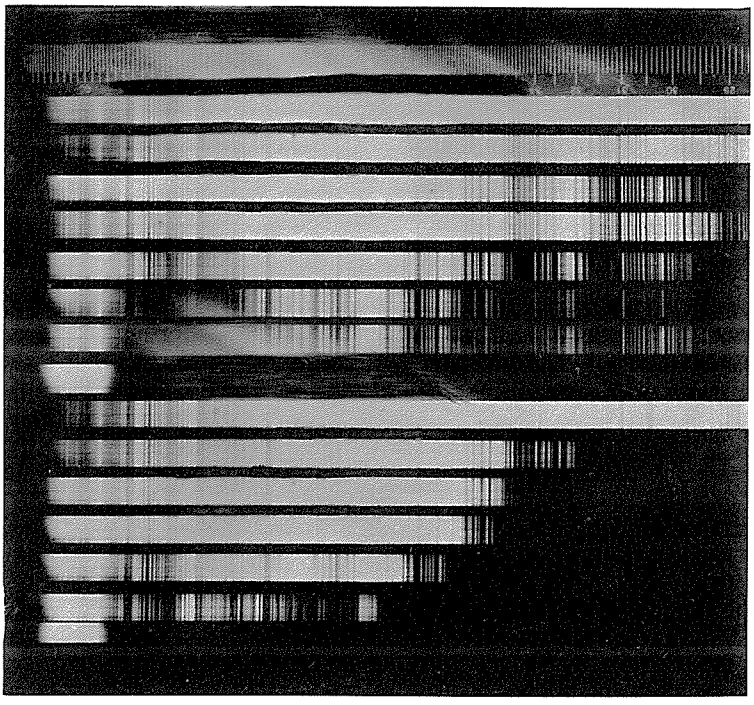
Phot. F′.



Phot. G.

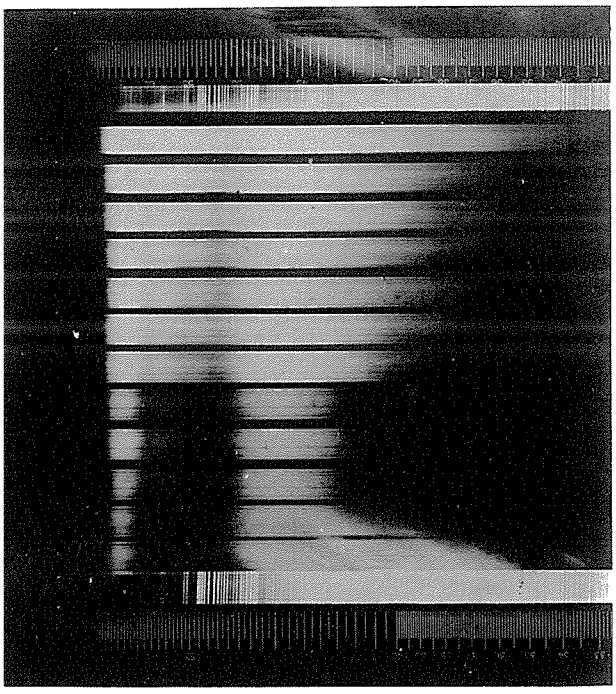
Phot. H.

Histidin.



Tyrosin.

Phot. J.



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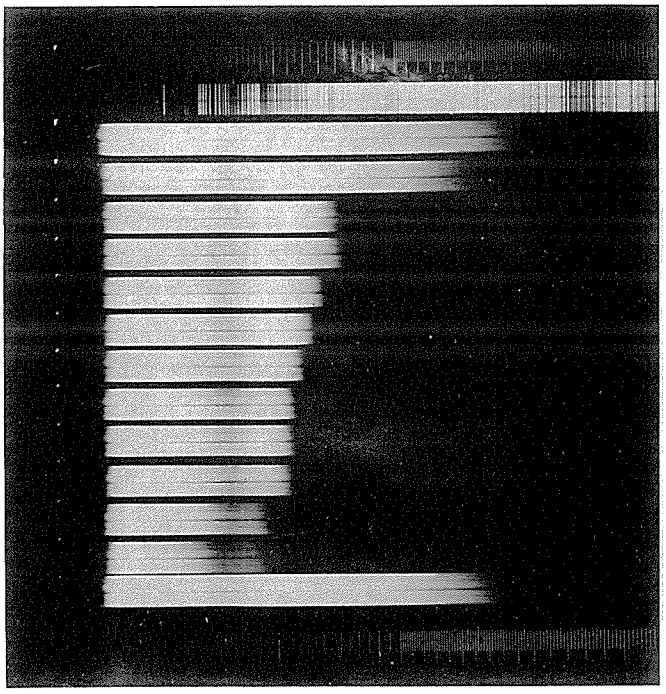

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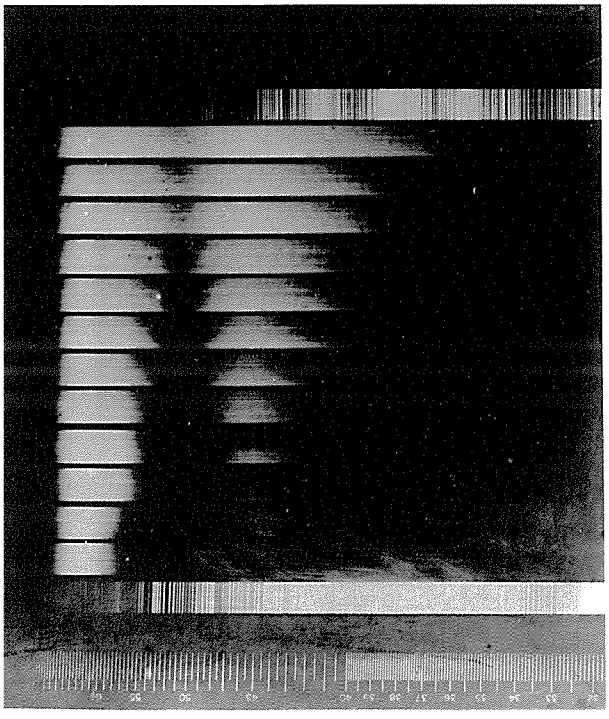

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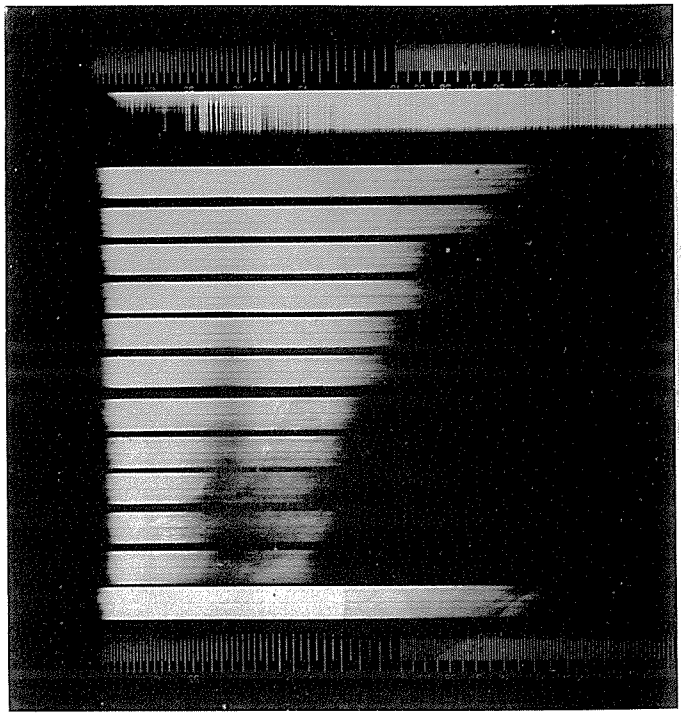

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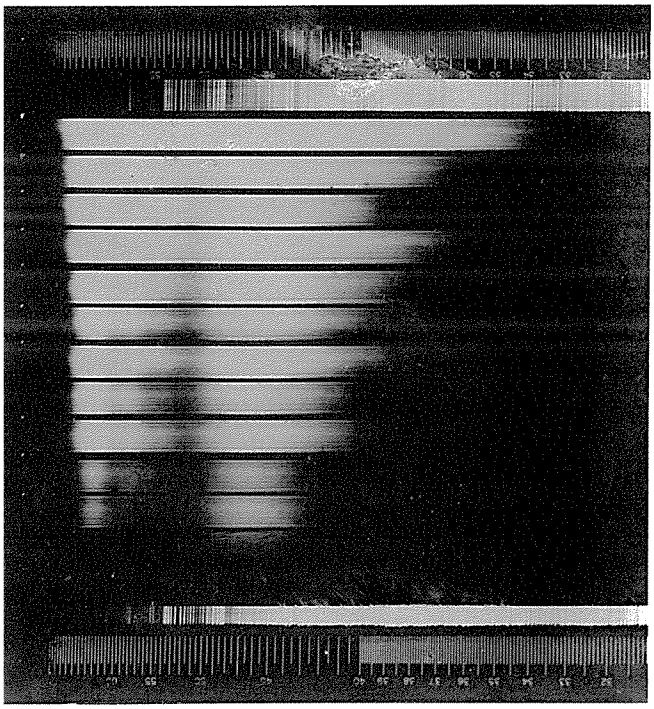

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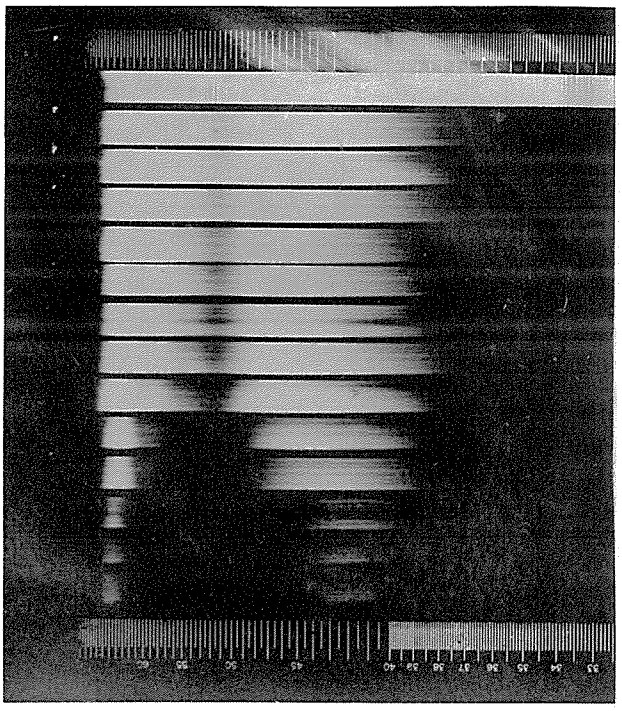
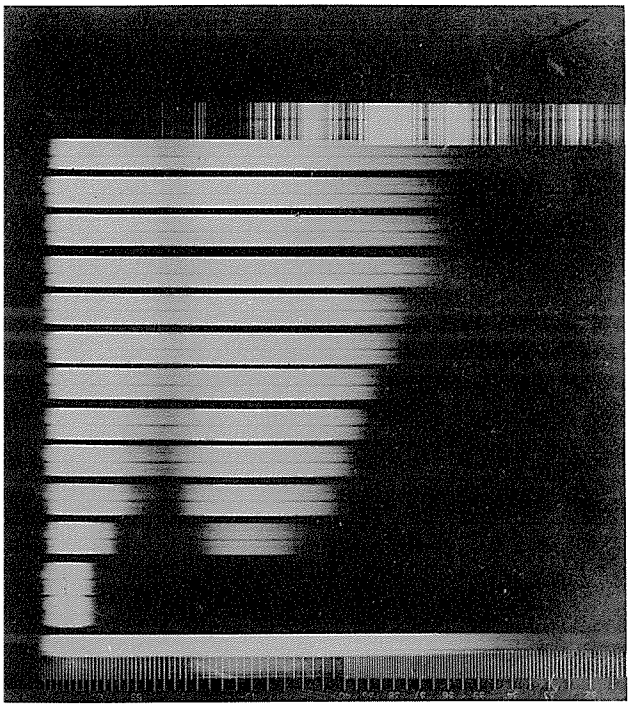
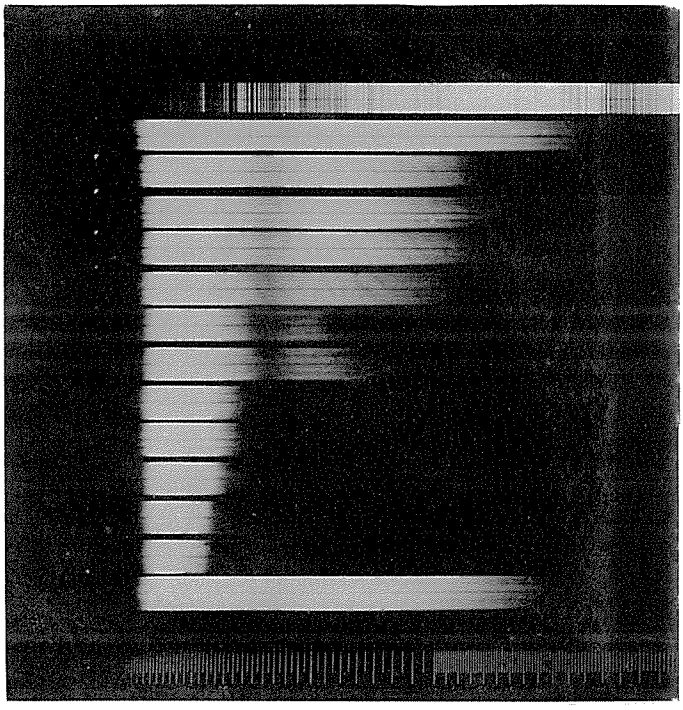


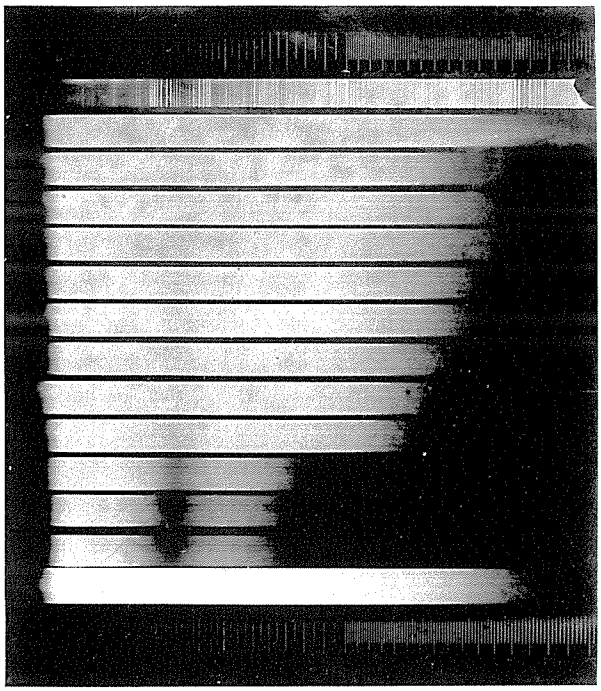
Phot. O_A.



Phot. O_B.



Phot. Q_A.



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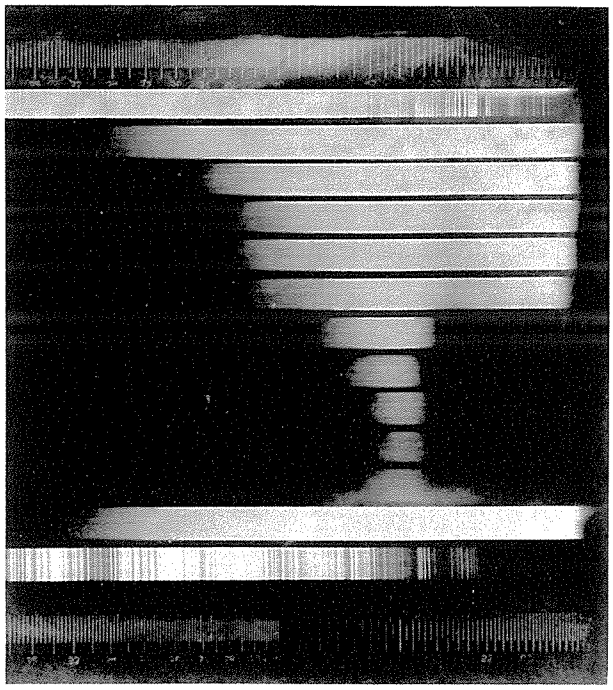

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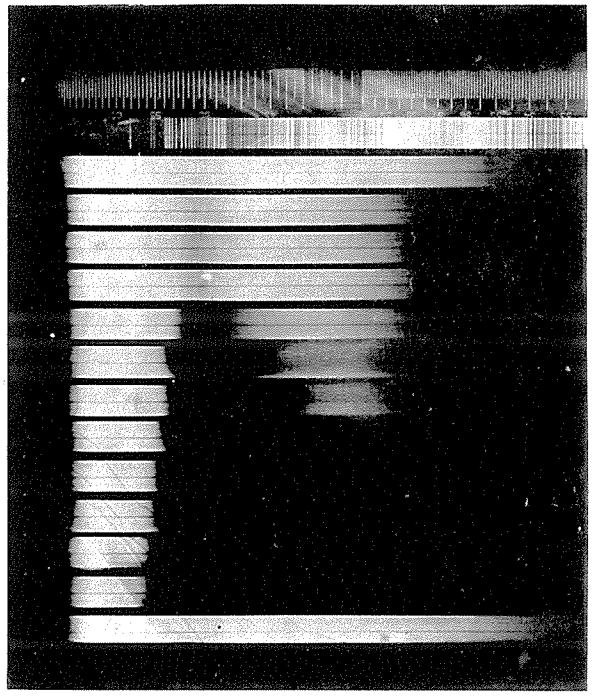
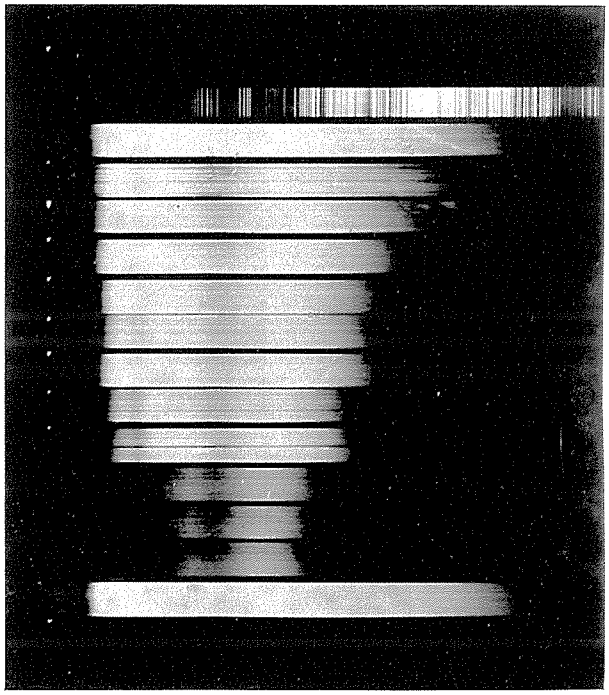
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