dissolved in 25 cc. of water and 75 cc. of ethyl alcohol. The mixture was allowed to stand for nine days and the crystalline precipitate then collected on a filter. The crude product melted at 138–140° and after crystallization from alcohol melted at 138–139°.

Anal. Calc. for $C_{16}H_{10}N_2$: N, 11.9. Found: 12.1.

Preparation of Benzoyl-cyclopropane.—Ten g. of chloroketone was mixed with a solution of 11.3 g. of potassium hydroxide in 130 cc. of methyl alcohol; after 18 hours, about half the alcohol was distilled and the residue diluted with water and extracted with ether. The ether was dried and distilled and the residual oil distilled under diminished pressure, passing over almost completely at 140–142° (37 mm.); yield, 5.5 g. Its identity as benzoyl-cyclopropane was established by the following experiments.

a. On treatment with semicarbazine hydrochloride and potassium acetate in alcohol at room temperature for 12 hours, a solid product was obtained which after crystallization melted at 182–183° (Kishner[6b] gives 185°).

b. Treated with hydroxylamine hydrochloride and potassium hydroxide in alcohol at room temperature, an oil was obtained which soon crystallized and after recrystallizing from petroleum ether melted at 90–94° (Perkin[6a] gives 90–92°; Kishner,[6b] 86–89°).

c. Bromine in carbon tetrachloride was without action on the substance except in the sunlight when the bromine color slowly disappeared and hydrobromic acid was evolved.

Summary

γ-Chloropropyl-phenylketone was synthesized by the action of phenylmagnesium bromide on γ-chloro-butyronitrile which can be prepared from trimethylene glycol without much difficulty. On treatment with potassium hydroxide in alcohol, the chloroketone is transformed into benzoyl-cyclopropane.

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[CONTRIBUTION FROM THE HARRIMAN RESEARCH LABORATORY, THE ROOSEVELT HOSPITAL AND THE HUNTINGTON FUND FOR CANCER RESEARCH, MEMORIAL HOSPITAL]

STUDIES ON ENZYME ACTION. XXVIII
THE SPONTANEOUS INCREASE IN THE ACTIVITIES OF LIPASE AND PROTEASE OF TISSUE EXTRACTS

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Introduction

It was shown in an earlier paper[1] that the sucrase activity of banana extracts on standing increased for a certain period of time and then decreased again. The increases amounted to 40 to 100% of the original activities, were independent of the compositions of the extracting solutions and of the preservative used, were not due to the presence of banana cells or bacteria, and were not accounted for by changes in hydrogen-ion concentration. The natures and amounts of increases were found, however, to be dependent upon the state of ripeness of the bananas when

[1] McGuire and Falk, THIS JOURNAL, **45**, 1539 (1923).

extracted. Willstätter and Racke[2] observed increases in sucrase actions in 14 out of 27 yeast extracts. These increases, while definite, were not large. Willstätter and Pollinger[3] found that a number of peroxidase preparations (from turnips) spontaneously increased in activity, at times as much as 40–50%. These increases occurred with both crude and purified preparations. In solution they occurred in several days or less; in dry powdered form increases were observed after months or even years.

In this paper, spontaneous increases in certain lipase and protease actions of some tissue and tumor extracts will be described.

Experimental Methods and Results

The tissue or tumor material was ground in a meat chopper, extracted with the requisite amount of water overnight, and filtered on paper. The extracts, cloudy at times, were tested for lipase and protease actions at once and at intervals thereafter, after standing at room temperatures, toluene being present throughout the experiments. For the lipase or ester-hydrolyzing tests, after proper dilution, 3.4 milli-equivalents of a number of different esters were added to 15cc. portions of extracts, incubated for 22 hours at 37°, and titrated with 0.1 *N* sodium hydroxide solution with phenolphthalein as indicator. For the protease actions, 0.1 g. portions (in solution for convenience of working) of a peptone, a casein and a gelatin preparation were added to the extracts to be tested and titrated by the formol method after similar incubations. The mixtures were all brought to *P*H 7.0 initially. Duplicate determinations were made in every case, and the necessary corrections for blanks introduced.

Table I shows the lipase actions and changes in terms of tenths of milli-equivalents of ester hydrolyzed (the actual titrations obtained corrected for blanks). Table II shows the results similarly for the protease actions

TABLE I

ESTER-HYDROLYZING ACTIONS OF TISSUE AND TUMOR EXTRACTS AFTER DIFFERENT TIME INTERVALS

	Expt. 120 whole rats, 3½ days old			Expt. 109 whole rats 22 days old			Expt. E10(A) rabbit livers (pregnant 24 days)			Expt. E7(C) rabbit livers (pregnant 28 days)	
Days	0	8	16	0	3	9	0	10	20	0	15
Phenyl acetate.....	4.30	4.03	4.08	6.06	5.94	5.97	4.38	3.47	3.26	4.56	2.88
Glyceryl triacetate..	2.72	2.94	2.92	3.71	3.35	3.28	3.10	2.99	2.66	3.06	2.78
Methyl butyrate...	1.31	1.73	1.68	2.62	3.55	3.20	4.66	4.57	4.29	3.63	3.72
Benzyl acetate.....	0.79	0.94	0.88	1.26	1.68	1.67	1.94	1.98	1.67	1.78	1.96
Ethyl acetate.....	.78	.83	.81	1.24	1.39	1.40	2.11	2.08	2.03	1.23	1.48
Methyl acetate.....	.73	.74	.68	1.05	1.16	1.05	2.25	2.59	2.06	1.60	1.80
Ethyl butyrate.....	1.40	1.75	1.62	2.69	3.55	3.37	3.66	4.02	3.47	2.64	3.05
Methyl benzoate...	0.23	0.20	0.21	0.68	0.61	0.61	1.16	1.18	0.97	0.82	0.82
Ethyl benzoate.....	.36	.36	.33	.77	.82	.82	1.17	1.11	0.97	.54	.55
*iso*Butyl acetate....	.80	.94	1.00	1.33	1.70	1.66	1.89	2.15	1.80	1.79	1.88

[2] Willstätter and Racke, *Ann.*, **427**, 111 (1922).

[3] Willstätter and Pollinger, *Ann.*, **430**, 269 (1923).

TABLE I (*Concluded*)

Days	Expt. E10(C) rabbit lungs (pregnant 24 days) 0	24	Expt. 114 Bashford mouse carcinoma 0	18	Expt. R34 Human fibromyoma of uterus 0	12	21	28	Expt. R38 Human fibromyoma of uterus 0	13	23
Phenyl acetate.....	2.68	2.06	2.38	2.29	1.62	2.04	2.19	2.51	2.01	1.92	1.92
Glyceryl triacetate.	1.53	1.42	1.95	2.19	1.30	1.29	1.37	1.25	1.32	1.15	1.06
Methyl butyrate...	2.31	2.54	1.47	1.94	2.72	4.16	4.50	4.49	2.42	2.72	2.88
Benzyl acetate.....	0.93	0.95	1.83	2.08	0.60	0.60	0.52	0.38	0.40	0.41	0.38
Ethyl acetate.....	.97	1.11	0.80	0.82	1.41	1.73	1.88	1.75	1.06	1.04	.96
Methyl acetate.....	1.14	1.41	..	..	2.14	2.00	1.95	1.90	1.49	1.25	1.05
Ethyl butyrate.....	2.20	2.52	..	..	2.93	3.81	4.15	..	2.25	2.52	2.55
Methyl benzoate...	0.73	0.79	..	..	0.83	1.05	1.17	..	0.63	0.74	0.59
Ethyl benzoate.....	.69	.72	..	..	.86	0.90	0.98	..	.64	.68	.66
*iso*Butyl acetate....	1.03	1.10	..	..	.77	.70	.58	..	.44	.46	.33

TABLE II

PROTEASE ACTIONS OF TISSUE AND TUMOR EXTRACTS AFTER DIFFERENT TIME INTERVALS

Expt.	Days	Peptone	Casein	Gelatin
120	0	1.63	2.31	0.74
Whole rats $3^1/_2$ days old	8	1.49	2.47	.61
	16	1.33	1.95	.70
119	0	1.61	1.72	.65
Whole rats 4 days old	12	1.17	2.06	.78
113	0	1.05	2.60	1.91
Whole rats 28 days old	6	1.05	3.17	2.12
	17	1.09	2.85	2.02
	26	0.88	2.96	1.88
	33	.96	2.74	1.89
E9G	0	1.12	0.47	0.19
Rabbit livers (pregnant 23 days)	7	0.93	.86	.25
	17	.70	.95	.16
	28	.69	.38	.12
E10A	0	1.10	1.07	.26
Rabbit livers (pregnant 24 days)	10	0.85	1.24	.20
	20	.73	0.52	.19
E10B	0	.42	.39	.13
Rabbit leg muscle (pregnant 24 days)	10	.35	.75	.11
	20	.14	.08	.04
R34A	0	.79	.69	.15
Human fibromyoma of uterus	12	.81	.89	.27
	21	.91	1.32	.37
R37A	0	.63	0.69	.25
Human fibromyoma of uterus	12	.51	.89	.22
	19	.54	1.06	.24
R37C	0	.73	.72	.37
Human fibromyoma of uterus	12	.55	.99	.25

in terms of total titrations, that is, the sums of the titrations before and after the addition of neutralized formaldehyde.

The concentrations of the various extracts were not the same but were

varied depending upon the enzyme activity, although for any one extract and series they were constant. Calculated in terms of milligrams of tissue or tumor extracted per cubic centimeter of solution as tested, they were 17.8 mg. in Expts. E10 liver, lung and leg muscle, E7, E9 and 114; 26.7 mg. in Expts. 113 and 119; 29.4–29.6 mg. in Expts. 109 and 120; and 88.9 mg. in Expts. R34, R37 and R38.

Discussion of Results

In the consideration of the experimental results, the possible experimental error is of importance. This may be placed at 0.1–0.2 for the various tests as judged from a large mass of data, so that differences of action of less than 0.2 cc. cannot be looked upon as significant.

The more striking features of the results given in the tables can be summarized briefly. In the first place it must be stated that not all extracts tested gave increases; only a limited number were found to do so. Up to the present it has been impossible to predict the behavior of a definite extract. Practically all except those given here showed gradual decreases in action.

For the lipase actions, the significant fact appears that some of the ester hydrolyses were increased, in fact doubled at times, others were unchanged, and others decreased more or less rapidly. In general terms where increases were observed, it was found to be with the butyric esters. All tests were made under the same conditions of hydrogen-ion concentration, temperature, etc., so that these selective increases are unquestioned. Similar relations were found with the protease actions, although only three protein preparations were studied. If increases were observed, it was generally with the casein, the actions on the gelatin were smaller as a rule and showed no changes, while the peptone actions mostly decreased with time. The increases in the lipase and protease actions which were observed reached a maximum in most of the tests and then began to decrease again.

The possible changes which are manifested in the spontaneous activations described here and elsewhere, may be considered to consist either in the decomposition or change of some substances present in the materials by which active enzyme groupings or active enzyme molecules are formed, or the breaking up of compounds of enzymes and inactivating materials which mask the enzyme actions, by which the latter are removed and active enzymes produced. Either process would be a chemical transformation accompanied by the appearance of new or additional enzymes.

The spontaneous increases in various enzyme actions including lipase, protease, sucrase and peroxidase, indicate a common property involving enzyme formation or production in the absence of a life process and only

from materials obtained directly from the living matter. They are of interest as showing possibilities of increases in enzyme activities, at present uncontrolled, which have been assumed, perhaps tacitly, to occur only during life processes. Decreases in enzyme actions are too common to consider in this connection, but such increases as are described compel attention. The selective increases in the lipase and protease actions indicate that the characters of the enzymes are changing and may perhaps parallel or correspond to changes in substances in the growth of living organisms. In general, the bearing of these relations upon the mechanism of the chemical transformations which occur in life processes is of interest.[4]

Summary

Spontaneous increases in the lipase and protease actions of a number of tissue and tumor extracts are described. These increases were selective in character, taking place with certain substrates and not with others under the same conditions. The possible significance of such changes in action in connection with phenomena occurring in life processes is indicated.

NEW YORK, N. Y.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

THE USE OF ALIPHATIC ACID ANHYDRIDES IN THE PREPARATION OF KETONES BY THE FRIEDEL AND CRAFTS REACTION

BY C. R. NOLLER[1] WITH ROGER ADAMS

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The use of acid anhydrides in the Friedel and Crafts reaction has been limited very largely to phthalic anhydride or its substitution products. This has come about because of the ease of conversion of the benzoylbenzoic acids obtained into anthraquinones in such yields as to make the process of practical importance. The details which have been developed in connection with the synthesis have shown that two or more mols. of anhydrous aluminum chloride must be employed if a high yield of benzoylbenzoic acid is to be obtained.

Considerably less study has been devoted to the condensation of succinic anhydride and maleic anhydride with aromatic compounds by means of anhydrous aluminum chloride. Here also, however, two or more mols. of condensing agent must be used in order to produce satisfactory yields.

[4] Compare Falk, "Catalytic Action," The Chemical Catalog Co., Inc., New York, 1922; Chapter VII, "A Chemical Interpretation of Life Processes."

[1] This communication is an abstract of a portion of a thesis submitted by C. R. Noller in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry at the University of Illinois.