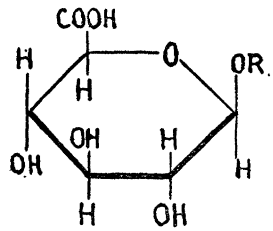
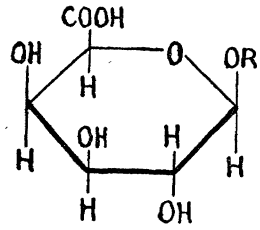
experiments one is faced with the necessity of explaining a new and peculiar fact, namely, the reason underlying the protective action exhibited by the antisera elicited by antigens containing isomeric aldobionic acids against infection with the same microorganism. It is possible to demonstrate experimentally that the protective action of cellobiuronic and gentiobiuronic acid antisera can be attributed to the antibody evoked by the glucuronic acid component common to the two aldobionic acid antigens.

Two artificial antigens, one containing the azo benzyl glycoside of glucuronic acid, the other that of galacturonic acid, have been prepared. The amino benzyl glycosides of these two uronic acids differ only in the configuration of the fourth carbon atom where the position of the H and OH groups is interchanged, as can be seen in Figs. III and IV.



p-aminobenzyl glucoronide
R = $NH_2C_6H_4CH_2$–
Fig. III



p-aminobenzyl galacturonide
Fig. IV

This alteration in constitution has a profound influence in determining the serological specificity of these antigens, as was shown in a study from this laboratory several years ago.[5] That this alteration in configuration of but one carbon atom will determine the capacity of the hexuronic acid antigens to evoke antibacterial immunity is likewise evident, for it has now been found that the antisera of rabbits injected with the glucuronic acid antigen protect mice against infection with 100,000 minimal lethal doses of Type II pneumococci (though not to Types III and VIII), whereas the antiserum to the galacturonic acid antigen is devoid of any protective action whatsoever.

In view of this experimental evidence it is justifiable to conclude, first, that passive immunity to Types III and VIII pneumococcal infection can be conferred on mice by the sera of rabbits immunized with an artificial antigen containing an aldobionic acid having an exact and appropriate configuration; second, that it is possible to confer passive immunity to Type II pneumococcal infection on mice with the sera of rabbits injected with artificial antigens containing saccharides of synthetic origin. The immunity conferred appears to be attributable to an antibody directed toward glucuronic acid. If the molecular configuration of but one of the carbon atoms of the hexuronic acid constituent of the antigen is altered, its capacity to evoke immunity to Type II pneumococcal infection is lost.

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[5] W. F. Goebel and R. D. Hotchkiss, *Jour. Exp. Med.*, 66: 191, 1937.

THE DETERMINATION OF THE TOTAL D-AMINO ACID CONTENT OF HUMAN TUMORS AND NORMAL TISSUES BY MEANS OF D-AMINO ACID OXIDASE

THE reports in recent months by Kögl and co-workers[1] of the characteristic occurrence of amino acids of unnatural configuration in malignant tumors in man and rabbit have been followed by several papers[2] tending both to confirm and to discredit this claim of obviously profound and immediate interest for the biochemistry of tumors. Most of the reports concerned make evident certain difficulties involved in establishing or rejecting such a conclusion on the basis of isolation procedures. We have, therefore, utilized the specific *d*-amino acid oxidase preparation of Krebs[3] for the determination of the *total d*-amino acid content of hydrolysates of a fairly representative variety of benign and malignant human tumors, normal human tissues and some proteins of especial interest: the urinary Bence-Jones protein, because of its close correlation with multiple myeloma in man; insulin as a hormone; and gliadin because of its very high (45 per cent.) glutamic acid content. The hydrolyses were conducted essentially as described by Kögl, in initially 25 to 35 per cent. HCl for 7 to 15 hours, usually with 10 gm acetone-dried tissue, or occasionally directly with fresh tissue. After elimination of the excess HCl, and appropriate neutralization and dilution, the *d*-amino and total nitrogen contents of aliquot portions were determined as reported in Table 1, which gives all data of this nature obtained by us to date.

All biologically significant *d*-α-amino acids are oxidatively deaminated by the Krebs enzyme, but at widely varying rates, so that to obtain reasonably complete oxidation of the slowly reacting *d*-amino acids the experiments were run for 20 hours with concentrated enzyme preparations stabilized by gum ghatti, and with thymol crystals added as antiseptic. As indicated in the table, 70 to 80 per cent. of the *d*-glutamic acid, the slowest reacting amino acid (*cf.* Krebs, 3,

[1] F. Kögl and H. Erxleben, *Verh. Kon. Ned. Akad. Wet.*, II, 38: 1, 1939; *Z. physiol. Chem.*, 258: 57, 1939, 261: 154, 1939; *Naturwissensch.*, 27: 486, 1939; *Nature*, 144: 111, 1939; F. Kögl, *Klin. Wochensch.*, 18: 801, 1939; *Z. f. Krebsforsch.*, 49: 291, 1939; F. Kögl, H. Erxleben and A. M. Akkerman, *Z. physiol. Chem.*, 261: 141, 1939.

[2] (Confirmatory) L. E. Arnow and J. C. Opsahl, SCIENCE, 90: 257, 1939; J. White and F. R. White, *Jour. Biol. Chem.*, 130: 435, 1939. (Non-confirmatory) A. C. Chibnall, M. W. Rees, G. R. Tristam, E. F. Williams and E. Boyland, *Nature*, 144: 71, 1939; S. Graff, *Jour. Biol. Chem.*, 130: 13, 1939; E. Chargaff, *Jour. Biol. Chem.*, 130: 29, 1939; C. Dittmar, *Z. f. Krebsforsch.*, 49: 397, 444, 1939.

[3] H. A. Krebs, *Biochem. Jour.*, 29: 1620, 1935.

TABLE 1

THE MAXIMUM *d* AMINO ACID CONTENT OF HYDROLYSATES OF HUMAN TUMORS, TISSUES AND PROTEINS DETERMINED BY MEANS OF *d*-*a*-AMINO ACID OXIDASE

Column 1	2	3	4	5	6	7
Material	Source	cmm O_2 consumed (exp. obs.[a])	mg *d*-N calc.[b] per cc[c]	mg total N per cc[c]	Per cent. *d*-N of total N	(Per cent. recovery of added *d*-N)
Protein	E. Bence-Jones	19; 20	0.041	7.13	0.6	
	M. Insulin	59	0.074	4.76	1.5	
	L. Gliadin	35	0.073	5.71	1.3	70 (*d*-glut-N)[d]
Normal Tissue	B. Liver	58	0.120	5.04	2.4	79 "
	H. Lung	28; 27	0.058	5.14	1.1	
	Y. Breast	66; 67	0.014	.78	1.8	
	P. Spleen	97	0.244	11.66	2.1	
Benign Tumor	Z. Ovary (fibroma)	64	0.016	.90	1.8	
Sarcoma	J. Back (fascial and liposarcoma)	36	0.076	3.42	2.2	82 "
Carcinoma	D. Liver (secondary from thyroid)	37	0.080	4.66	1.7	71 "
	N. Liver (secondary from rectum)	137	0.226	14.61	1.5	
	F. Ovary (secondary from uterus)	28; 29	0.060	5.20	1.2	
	K. Breast (infiltrating duct, gr. 4)	27	0.056	4.67	1.2	87 "
	X. Breast " " gr. 2)	47; 45	0.096	2.62	3.7	
Miscellaneous Malignant	G. Kidney (Wilms' adenomyosarcoma)	22; 25	0.049	5.12	1.0	
	I. Abdomen (secondary teratoma testis, mainly carcinoma)	31	0.065	4.64	1.4	80 "
Leukemia	O. Spleen (myelogenous, acute)	84	0.104	4.89	2.1	
d,l-glutamic acid		79; 89; 89		0.280	36; 40; 40	71; 80; 80
d,l-alanine		109; 119		0.280	48; 53	97; 106
d-phenylaminobutyric acid		272		0.350	97	97
amino acid mixture[e] approximating casein		211		1.30	20	92
l-glutamic acid		6		1.40	(0.6)	

F,J,K,X,Y,Z, Memorial Hospital Nos. 1968, 1471, 2062, 1255, 1287 (operative); B,D,G,H,I, Nos. 1496, 1496, 1699, 1496, 2052 (autopsy). N,O,P, New York Hospital Nos. 9759, 9762, 9764 (autopsy). Normal tissues B,H,Y, from tumor cases; P from non-tumor case.

[a] With samples of diluted neutralized hydrolysate, varying from 0.6 to 6 cc.

[b] Cmm $O_2 = 0.00125$ mg N ($RCHNH_2COOH + \frac{1}{2} O_2 = RCOCOOH + NH_3$).

[c] Per cc of neutralized hydrolysate diluted variously 1/10, 1/25, or 1/100.

[d] "112" cmm *d*-glutamic acid (as *d*-*l*-) added to replicate, diluted hydrolysate aliquots of B,D,I,J,K,L, immediately before analysis by d-amino acid oxidase; recovery calculated from difference between replicates ± d-glutamic-N addition.

[e] 17 amino acids[4] containing 8 in *d*-*l*-form (alanine, valine, leucine, isoleucine, phenyl-alanine, serine, lysine and threonine), and with 22 per cent. of the total amino-N in the *d* form.

Table XII), was recovered by the method and reaction period employed (100 per cent. of *d*-alanine or *d*-phenylaminobutyric acid, but a negligible quantity of *l*-glutamic acid). Seventy to 80 per cent. of *d*-glutamic acid was likewise analytically recovered when added to various samples before hydrolysis (sample D) or after hydrolysis (samples B, D, I, J, K, L, Column 7, and sample N[5]). The values in Column 3 have been corrected for any O_2 consumption by the enzyme preparation without substrate. Ammonia estimations were carried out in a number of cases in order to ascertain that the O_2 consumption was due mainly to *d*-amino acid oxidation. Values of equivalent NH_3 as much as 25 to 50 per cent. less than the O_2 consumption were sometimes obtained, suggesting concomitant oxidation of a certain amount of non-nitrogenous material; on the other hand, the NH_3 determinations were definitely less accurate than those of O_2 consumption, owing to the relatively large blank values for NH_3 in the hydrolysates themselves. The non-specific oxidation certainly more than counterbalanced the slight incompleteness of reaction caused by the more slowly reacting amino acid. The *d*-amino acid-N values in Column 6 are to be regarded, therefore, essentially as *maximum values*, perhaps 25 to 50 per cent. too high in some cases. For the question of malignancy specificity at issue, however, the two possible errors indicated are of little moment, since we are mainly concerned here with the order of magnitude of *d*-amino acid-N. It must be considered, furthermore, that the values in Column 6 include the amino acids already generally known to racemize partially on hydrolysis (serine, proline, cystine, etc.). Finally, it is to be observed that the materials reported in Table 1 are equivalent in number and variety to those reported by Kögl, namely, 7 normal tissues, 3 normal tissues from tumor-bearing animals 4 benign tumors and 12 malignant tumors.[1] We would likewise emphasize that the findings in our various types of control experiments lend great confidence as to the efficacy and adequacy of the simple, direct and specific enzymatic method of analysis employed, in contrast to the isolation procedures of Kögl involving incomplete and very possibly unrepresentative recoveries of enantiomorphs.

[4] V. du Vigneaud, J. P. Chandler, A. W. Moyer and D. M. Keppel, *Jour. Biol. Chem.*, 131: 57, 1939.

[5] Not added immediately before manometric analysis, as with samples B, D, I, J, K, L, but to strongly acidified, aliquot sample N hydrolysate mother liquor 39 days before eventual dilution and analysis, during which period two crops of practically pure *l*(+)-glutamic acid hydrochloride were removed (410 mg from initial 6.3 gm dried liver tumor, or 5.3 per cent. *l*-glutamic acid). The isolated second crop (260 mg) was shown by means of *d*-amino acid oxidase to contain no *d*-amino acid.

CONCLUSION

The uniformity and low order of *d*-amino acid

nitrogen observed in the variety of hydrolysates examined, whether from benign or malignant[6] tumors, normal tissues or proteins (Column 6), shows that malignancy is, *as a generality,* not specifically characterized by the presence of amino acids of unnatural (*d*-) configuration. Whatever interest certain of Kögl's data may retain for general biochemistry, the main contention concerning malignancy specificity is, for the cancer field, evidently no longer tenable.

Further experimental details and discussion of our work will be forthcoming, together with accompanying data on glutamic acid isolations from some of the more interesting hydrolysates. Following the suggestion of Professor du Vigneaud, most of the hydrolyses were carried out in HCl containing heavy water, and experiments to determine the content of deuterium attached to the *alpha* carbon atom will be performed with any *d*-glutamic acid found. It is hoped that this procedure will provide a critical decision as to whether such glutamic acid as may occur partially racemized in the hydrolysates had been formed during the process of hydrolysis.

We are deeply indebted to Dr. C. P. Rhoads, Dr. Fred Stewart, Dr. Frank Foote, Dr. Ruth Snyder and Dr. John Pool, of Memorial Hospital, for large numbers of tumors and tissues identified and kindly placed at our disposal by them; likewise to Dr. Jacob Furth, of the Department of Pathology, Cornell University Medical College and New York Hospital; to Miss Betty Robinson for technical assistance; to the National Advisory Cancer Council, the Jane Coffin Childs Memorial Fund, and the Ella Sachs Plotz Foundation for grants; and, finally, to Professor Vincent du Vigneaud for his counsel.

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[6] The rather higher value for sample X (3.7 per cent. *d*-N) was not obtained with the other primary breast carcinoma of even more advanced malignancy (sample K, 1.2 per cent. *d*-N).

[7] Lalor Foundation Fellow.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLE METHOD TO USE IN RECOVERING FECES AT MEASURED INTERVALS

IT is often necessary to collect fecal droppings from test animals at given intervals, and to do this by means of a simple, inexpensive and yet accurate mechanism would facilitate the handling of many physiological problems. This is especially desirable where constant attendance is not possible and a continuous record is desired. Such is the case when measuring the time at which poisons are eliminated from an animal body.

The method, as outlined below, was used to accurately determine the larval life history of *Pieris rapae* (Lepidoptera), and could be used in toxicological investigations on many leaf-feeding insect larvae.

By connecting the hour-hand mechanism of a clock to a thin aluminum disk (a phonograph record may be equally well used, depending upon the chemical requirements desired) supported on a bearing so as to rotate freely, the disk will revolve once in every twelve hours. An insect feeding upon a small leaf suspended above the moving circular sheet will leave, by means of the excrement dropped, a record of its activities. If ingested poisons caused the insect to fall, it would remain on the record as its own marker of the time of collapse. With *Pieris rapae,* the insect fed on a rooted cabbage leaf suspended in a constant temperature compartment over the disk which provided an adequate time-recording mechanism and left its whole history in the form of pellets and cast skins.

Numerous adaptations may be applied. With caged animals such as mice, a funnel placed under wire-floored cages can direct excreta to a series of pockets on the circular sheet, and with a series of removable disks a semi-permanent record is obtained which may later be analyzed. A record of the number of caterpillar pellets may be plotted with time as the abscissa and number of pellets as the ordinate, and the life history with molts can be timed with far less experimental error than is introduced by normal variation. Should it be desirable to run more than twelve hours without attention, a large disk can be used and the speed geared down; or, as was done in one instance, the source of pellets was shifted into another circle by means of an electrical circuit being completed by contact to a point on the disk, so as to act through a relay.

In insects at least, the amount of excreta is proportional to the feeding and may be used to investigate the effect of factors such as light, temperature, humidity, repellents, etc., on the feeding rate. With *Pieris rapae* it was found that (1) the number of pellets excreted, if grouped in hour or two-hour intervals, will show pronounced fluctuations, there being periods of greater or lesser feeding. (2) The length of the non-feeding periods during ecdysis one through four were successively 9, 16, 17 and 29 hours. (3) The feeding gradually builds up to a maximum in each instar and then abruptly ceases in preparation for the molt. (4)