Cysteine would be available for this reaction and felinine might be the non-toxic end product. On the other hand the new amino acid could be a normal product of mammalian metabolism and take part in the transfer of C_5 units (i.e. isoprene-type) or C_3 units (isopropyl units found by oxidation and removal of C_3 as acetate) in the synthesis of such substances as sterols or carotenes which require a branched-chain carbon skeleton. Whilst such a substance need, in most animals, occur only transiently, it is possible that in the cat, owing to some specific peculiarity, this substance is accumulated and excreted.

The C/2 substance which is formed on treatment with acid is not believed to occur naturally. No trace of it was observed on the two-way paper chromatograms of cat urine and none was encountered in the course of the large-scale fractionation.

The structure proposed for felinine must remain provisional until further proof becomes available. Some attempts have already been made to synthesize the new amino acid and also the C/2 substance derived from it. This work will be reported in a later paper.

SUMMARY

1. A new sulphur-containing amino acid (felinine) has been isolated from cat urine following a series of fractionations on ion-exchange resins.

2. This substance is excreted by the cat in amounts as high as 100–120 mg./day, which is greater in amount than that of any other single amino acid.

3. Degradative studies with Raney nickel led to the identification of alanine, isoamyl alcohol and hydrogen sulphide indicating that felinine is a *S*-hydroxyalkylcysteine, the alkyl group having the carbon skeleton of isopentane.

4. On treatment with mineral acid felinine gives rise to a second sulphur-containing amino acid (C/2), which is believed to be a dicysteine derivative. This substance does not occur as such in cat urine.

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REFERENCES

Bloch, K. (1944). <i>J. biol. Chem.</i> 155, 255. Bougault, J., Cattelain, E. & Chabrier, P. (1938). <i>Bull. Soc. chim. Fr.</i> [5], 5, 1699. Crumpler, H. R. & Dent, C. E. (1949). <i>Nature, Lond.</i>, 164, 441. Crumpler, H. R., Dent, C. E., Harris, H. & Westall, R. G. (1951). <i>Nature, Lond.</i>, 167, 307.	Datta, S. P. & Harris, H. (1951). <i>J. Physiol.</i> 114, 39P. Dent, C. E. (1947). <i>Biochem. J.</i> 41, 240. Dent, C. E. (1948). <i>Biochem. J.</i> 43, 169. Dent, C. E. & Harris, H. (1951). <i>Ann. Eugen., Lond.</i>, 16, 60. Partridge, S. M. (1948). <i>Biochem. J.</i> 42, 238. Partridge, S. M. & Brimley, R. C. (1951). <i>Biochem. J.</i> 48, 313. Westall, R. G. (1952). <i>Biochem. J.</i> 52, 638.

A Chemical Examination of Connective Tissue in Rheumatic Fever

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A characteristic feature of subcutaneous nodules in rheumatic fever and also in rheumatoid arthritis is the central core of eosinophil fibrillar material, usually termed 'fibrinoid' from its histological resemblance to fibrin. However, there is much conflict of opinion regarding the presence of fibrin in such lesions, and these opinions are based mainly on histological grounds (cf. Glynn & Loewi, 1952), and few chemical or physical studies have been made on such tissue. Much stronger evidence for the presence of degraded collagen in rheumatoid arthritis is

afforded by electron-micrograph and X-ray studies of Kellgren, Ball, Astbury, Reed & Beighton (1951), and chemical analyses of rheumatoid nodules have shown that the collagen present has its normal content of hydroxyproline (Bien & Ziff, 1951). The purpose of the present investigation was to examine chemically rheumatic fever nodules in order to ascertain what difference, if any, existed between them and normal connective tissue. A preliminary report of this work has been given by Consden, Glynn & Stanier (1952).

METHODS

The methods used were first chromatographic, which gave an overall picture of the amino-acid composition; and secondly, quantitative analysis of some of the amino acids and other constituents. As only a limited amount of tissue was available, and since this contained a mixture of different proteins, the analysis was restricted to those amino acids which would provide the most information. Therefore, those estimated were hydroxyproline, tyrosine and tryptophan. Since collagen is rich in hydroxyproline, and elastin is the only other animal protein which contains it in significant quantities, determination of this amino acid, after suitable fractionating procedures, provides an estimate of these two proteins, as has been shown by Neuman & Logan (1950*b*). Conversely, tryptophan, which is absent from collagen and allied proteins, is present in most other proteins, and estimation of this amino acid will, therefore, give a measure of protein constituents other than collagen and elastin. Tyrosine occupies an intermediate position, being present in only small amounts in collagen and elastin, but in much higher amounts in other proteins, especially in those of the blood. In addition to these amino acids, it was desirable to examine the carbohydrate constituents, since it is well established that polysaccharides occur in connective tissue.

Since nodules appear to contain a variable amount of unaltered collagen (Glynn & Loewi, 1952), it was desirable to separate this from non-collagenous material and to carry out analyses on both fractions. Collagen may be extracted by various methods, but it seemed preferable to use a mild method such as autoclaving with water at moderate pressure. The residue of connective tissue after autoclaving is usually regarded as being composed chiefly of elastin, though elastin from different sources does not always appear to be identical as regards amino-acid composition (Bowes & Kenten, 1949; Hall, 1951). Although collagen can be completely extracted by this method and elastin appears to be resistant, other insoluble proteins occupy an intermediate position. Nevertheless, this procedure affords a rough fractionation into a soluble collagen-rich and an insoluble non-collagen fraction. Both were analysed. Non-rheumatic subcutaneous tissue, from an area similar to that in which the nodules occurred, was also fractionated and analysed in order to provide a basis of comparison for the rheumatic tissue.

EXPERIMENTAL

Collection and pretreatment of tissues. Freshly excised, subcutaneous nodules from the elbow region were obtained from twelve rheumatic-fever patients of both sexes, whose ages ranged from 8 to 30 yr. Under the dissecting microscope, the nodules were freed as far as possible from adjacent, apparently normal, connective tissue. Part was retained for histological examination and the remainder was washed thoroughly with saline, then with water to remove salts and soluble proteins. After drying *in vacuo*, or alternatively, after dehydration by washing with successive portions of absolute ethanol, the tissue was extracted with benzene followed by light petroleum (b.p. 100–120°) to remove fatty material. The dried nodules thus obtained were small, ranging in weight from 1 to 15 mg. and were hygroscopic. A larger quantity of nodules was obtained *post mortem* from

a female child aged 8 yr. who had died from congestive cardiac failure during rheumatic fever. After the treatments described above the specimens were pooled for quantitative analyses; they weighed 135 mg.

Non-rheumatic tissue was obtained *post mortem*. After careful removal of any epidermis, the tissue was treated as described above. Two samples were obtained, one from a male aged 38 yr. who had died from a duodenal fistula, and the other from a male aged 9 yr. who had died from thrombosis of the sagittal sinus. The air-dry weights were 123 and 360 mg. respectively. Both these tissues did not differ, histologically, from normal. Healthy tendo Achillis was collected *post mortem* from a male aged 62 yr., cut into small pieces, and treated as described above. This served as a normal collagen control.

Histological examination. For this purpose material was fixed in 5% formol-saline, embedded in paraffin and sectioned at 5 μ . Sections were stained by a variety of techniques including haematoxylin and eosin, and Hotchkiss's periodic acid-Schiff reagent method for α -glycols. No nodule was used for chemical study unless typical 'fibrinoid' material was present on histological examination (Glynn & Loewi, 1952). The criteria for its recognition were intense eosinophilic amorphous and fibrillar material in the connective tissue giving a strongly positive reaction with the periodic acid-Schiff reagent and the presence of argyrophilic fibres in this material in sections stained by the silver impregnation method of Gomöri (1937).

Extraction of collagen. Tissues were autoclaved twice with water at 120° for 3 hr. in a sealed tube as described by Neuman & Logan (1950*b*). Extracts and washings were concentrated *in vacuo* to small bulk and finally dried *in vacuo* over H_2SO_4 . Original tissues, extracts and residues were weighed after drying to constant weight over H_2SO_4 in a vacuum desiccator. The tendon was completely extractable by autoclaving.

Chromatography. Samples were hydrolysed for 17 hr. at 100–110° with 6*N*-HCl, repeatedly evaporated *in vacuo* to remove excess acid, and finally made up to a suitable volume with water. Two-dimensional paper chromatography (Consden, Gordon & Martin, 1944), was carried out on the hydrolysates using an amount of solution equivalent to about 1 mg. of protein. In earlier experiments, the solvents were collidine for the first direction, and phenol:coal gas: NH_3 for the second, but for most of the chromatograms the phenol was replaced by water-saturated *m*-cresol containing 8-hydroxyquinoline (0.1%). With the *m*-cresol system a longer period of development is required (40 hr.) than for phenol (17 hr.). Except for aspartic and glutamic acids, which are barely separated, the *m*-cresol system gives a more satisfactory separation of the amino acids. The faster moving amino acids are more satisfactorily resolved, e.g. methionine is separated from the leucines, and arginine and lysine are better separated from each other. Histidine is clearly visible, and cystine, even if present in very small amounts, is usually distinctly visible. In some cases, the presence of cystine was confirmed by oxidizing a portion of the hydrolysate with bromine water in order to convert the cystine to cysteic acid (Consden & Gordon, 1950), followed by one-dimensional chromatography in phenol: NH_3 .

Examination of carbohydrates. The tissue was hydrolysed with $N-H_2SO_4$ at 100–110° for 3 hr., neutralized with $Ba(OH)_2$ and after separation from $BaSO_4$, concentrated so that 10 μ l. was equivalent to approx. 1 mg. of original substance. Sugars were examined by ionophoresis in

borate buffer at pH 8.6 according to the method of Consden & Stanier (1952*a*), in the apparatus described by Consden & Stanier (1952*b*), using 10–20 μ l. of hydrolysate. Further separation of sugars was carried out by subsequent chromatography at right angles to the direction of ionophoresis, using the *n*-butanol:acetic acid system of Partridge (1948), except that the developing solvent was only about 80% saturated with water in order to overcome the effect of the salt on the paper. For ionophoresis alone, Whatman no. 1 paper was used; the two-dimensional separations were carried out on Whatman no. 42.

Test for hexosamine. The above hydrolysates were used for this procedure. 150 μ l., equivalent to about 15 mg. protein, was applied along the starting line on a strip of Whatman no. 1 paper in the ionophoresis apparatus. Below this, and near one edge, was placed a control solution consisting of 5 μ l. hydrolysate to which was added about 40 μ g. glucosamine. The electrolyte solution consisted of 200 parts 0.67 M-acetic acid and 36 parts 0.67 M-Ba(OH)₂, its pH being 3.5. After 2 hr. at 10 v/cm., the paper was removed and dried. A strip containing the control was cut off and coloured with ninhydrin. The hexosamine spot occurred near the spots of lysine and arginine which had moved towards the cathode, whereas most of the ninhydrin-reacting constituents remained near the origin. By reference to the control strip, that portion of the remainder of the sheet containing the basic constituents was cut out and eluted with water. The eluate (1–2 ml.) was made acid to thymol blue with H₂SO₄, the precipitated BaSO₄ removed and acetic acid removed from the solution by repeated evaporation *in vacuo* with water. Excess H₂SO₄ was then removed by addition of Ba(OH)₂ to pH 5–6, the precipitate separated and the solution evaporated *in vacuo* to dryness. The solid was then dissolved in a small volume of water and transferred to a sheet of Whatman no. 4 paper for chromatography in *m*-cresol:hydroxyquinoline:NH₃ for 17 hr. Side by side with the test solution were placed the following control solutions: (a) glucosamine, (b) galactosamine, (c) a mixture of these two, and (d) an amino-acid mixture. Two chromatograms of controls and test solution were developed on the same sheet. After development and removal of solvent, the sheet was divided into two, so that each portion contained test and control solutions. One was treated with ninhydrin and the other with aniline hydrogen phthalate (Partridge, 1949). In this solvent system glucosamine is just separated from galactosamine, the former travelling at the rate of alanine and the latter at the rate of tyrosine. Both amino sugars with ninhydrin give grey and reddish-grey spots after a short heating period which gradually change to purple, galactosamine changing more slowly than glucosamine. In the test solution, strong spots of lysine and arginine occurred, but these were well separated from the hexosamine. However, relatively faint ninhydrin-reacting constituents occurred near the hexosamine positions, and were probably due to peptides. Aniline hydrogen phthalate was used as an additional test for the amino sugars, since amino acids and peptides do not react with this reagent, whereas the hexosamines behave similarly to the hexoses, both as regards the visible colour which is produced, and the fluorescence in ultraviolet light.

Quantitative analyses. Hydroxyproline was determined in the 6*N*-HCl hydrolysates by the method of Neuman & Logan (1950*a*). It was found that rather larger amounts of hydroxyproline were necessary for satisfactory colour development than suggested by these authors, a satisfactory

range being 0.025–0.075 mg. Tryptophan was determined by the method of Spies & Chambers (1948). For this analysis, samples were hydrolysed with 5*N*-NaOH at 100° for 17 hr. Tyrosine was estimated after hydrolysis *in vacuo* with 6*N*-HCl at 100–110° for 17 hr. by the method of Folin & Ciocalteu (1927). Ultraviolet spectrophotometric measurements of tyrosine and tryptophan (cf. Goodwin & Morton, 1946; Holiday, 1936) in the alkaline and acid hydrolysates were also undertaken and, although found to be not so consistent as the Folin-Ciocalteu method, there was fairly good agreement between the mean values determined by both methods. Nitrogen in samples of the hydrolysates was estimated by the micro-Kjeldahl method. Reducing sugars (as glucose) were estimated by the copper reduction method of Shaffer & Somogyi (1933). For this analysis the samples were hydrolysed with *N*-HCl at 100–110° for 3 hr. Hexosamine was estimated in the 6*N*-HCl hydrolysates by the method of Elson & Morgan (1933), there being insufficient material for the hydrolysis to be carried out under the milder conditions, which are usually employed for hexosamine determination. Prolonged hydrolysis with strong acid is known to destroy a little of the hexosamine (Johnston, Ogston & Stanier, 1951); on the other hand, in the presence of much protein, hexosamine figures may be erroneously high (Johns & Marrack, 1952), which we have confirmed in recovery experiments, with gelatin containing added amounts of glucosamine (1–2%). The 'protein' effect probably more than counterbalances the hydrolysis loss, and our hexosamine figures may be 5–10% too high.

RESULTS

Autoclaving. The twelve individual nodules left residues varying between 25 and 40% of the original weight. The pooled nodules from one case left 30% residue (Table 1). The residue of each of the two samples of non-rheumatic connective tissue left residues of about 10% (Table 1).

Chromatograms. All nodule extracts gave, after hydrolysis, chromatograms similar to those of hydrolysed extracts of non-rheumatic tissue. These resembled chromatograms of hydrolysed tendon and gelatin, except that somewhat more tyrosine was present in the tissue extracts. The chromatograms of the extracts therefore resembled that of collagen (Bowes & Kenten, 1949), which is characterized by the presence of hydroxylysine and of very strong glycine, hydroxyproline and proline spots. All nodule-residue chromatograms resembled each other but differed markedly from the non-rheumatic residue chromatograms. The latter were characteristic of elastin, e.g. from cattle ligamentum nuchae (cf. Bowes & Kenten, 1949) in which the strongest spots are those of glycine, alanine, valine and the leucines; hydroxyproline, aspartic and glutamic acids, the hydroxyamino acids and the basic amino acids are relatively weak. The nodule residues, however, gave chromatograms suggesting the presence of protein additional to elastin, since those amino acids which were weak in elastin were distinctly stronger and cystine, absent from elastin and non-rheumatic residues, was

Table 1. *Analysis of subcutaneous elbow tissue*

	Amount after autoclaving (%)	Chromatogram type	Hydroxy- proline (N as % total N)	Tyrosine (N as % total N)	Tryptophan (N as % total N)	Reducing sugar (as glucose, g./100 g. protein)	Hexosamine (g./100 g. protein)
Rheumatic fever, female, aged 8 yr.:							
Residue	30	Fibrin- elastin	0.7	3.4	0.8	3.6	0.9
Extract	70	Collagen	7.6	1.6	Trace	3.1	0.9
Non-rheumatic, male, aged 30 yr.:							
Residue	10	Elastin	1.1	2.5	—	—	—
Extract	90	Collagen	8.2	0.7	Trace	1.4	0.5
Non-rheumatic, male, aged 9 yr.:							
Residue	9	Elastin	0.9	3.5	—	2.3	—
Extract	91	Collagen	7.7	0.9	—	2.2	0.5
Comparative data:							
Gelatin*	—	—	8.6	0.4	0	0.2†	Trace†
Collagen*	—	—	8.0	0.4-0.6	0	0.4‡	0.2;‡ 0.3§
Elastin*	—	—	1.0-1.3	0.6-0.7	0	—	—
Human fibrin	—	—	0	2.7	2.7	4.5	1.1

* Data for amino-acid composition of bovine, bovine collagen and elastin (animal and human) from Tristram (1949), Bowes & Kenten (1949), Neuman & Logan (1950b) and Neuman (1949).

† Commercial bovine gelatin (present paper).

‡ Human tendo Achillis (present paper).

§ From Bowes (1951).

|| Amino-acid analyses, present paper; reducing sugar and hexosamine data from Consden (1953a).

usually present in small amount in the nodule residues. Chromatograms of the latter were similar to that of a mixture of fibrin and elastin and are therefore referred to as 'fibrin-elastin' (see Table 1), though it is not necessarily implied that only these two proteins are present.

Qualitative examination of carbohydrates. Nodules from several individuals were pooled, autoclaved and the fractions analysed for carbohydrates. The hexosamine in the extract was glucosamine; no galactosamine was found. This was true also of non-rheumatic tissue extract. The sugars present in hydrolysates of rheumatic and non-rheumatic residue and extract were found, by ionophoresis, to be mannose, galactose and glucose in addition to hexosamine; uronic acid was absent. In all the hydrolysates galactose was present in distinctly higher amount than the other two hexoses and glucose was relatively weaker than mannose. A two-dimensional separation (ionophoresis followed by chromatography) of a hydrolysate (1 mg. tissue) with added galactose and mannose (10 µg. each) was no different from that of the hydrolysate alone, except that the strengths of these two sugar spots were enhanced in the former case. A two-dimensional separation which is typical of all the hydrolysates is illustrated in Fig. 1.

Quantitative data. Quantitative data for the rheumatic-fever tissue from one individual and for the two non-rheumatic tissues are given in Table 1, together with comparative data for other proteins.

DISCUSSION

Chromatography and tryptophan and hydroxyproline figures suggest that both rheumatic and non-rheumatic extracts are similar to each other

Fig. 1. Two-dimensional separation of tissue hydrolysate on Whatman no. 42 paper. Ionophoresis in borate, pH 8.6 (3 hr. at 10 v/cm.) and chromatography in *n*-butanol:acetic acid (41 hr.) in directions shown. Aniline hydrogen phthalate spots: A, glucosamine; B, mannose (moderate-weak); C, galactose (strong); D, glucose (weak).

and are mainly collagen. In view of possible variations between individuals, the lower hydroxyproline of the rheumatic residue compared with that of non-rheumatic residue may not be significant.

However, if the hydroxyproline is due to elastin, then this protein amounts to 50–70 % of the nodule residue. Its tryptophan content would correspond to about 30 % fibrin, but a mixture of these two proteins in the above proportions would give a tyrosine figure of about 1 %, which is much lower than that actually found. Further, it was found that over 50 % of fibrin was extractable by autoclaving a preparation from a fibrin clot; therefore, if the nodule residue contained 30 % fibrin, an equal amount should have been present in the nodule extract. This would have made the tryptophan figure at least 0.3 % in the extract, whereas it was in fact much smaller. It would therefore appear that fibrin, if present in nodules, does not occur to the extent of more than a few per cent. The high tyrosine content of all the residues suggests the presence of a tyrosine-rich protein or polypeptide. However, neither of the methods used for tyrosine estimation is specific for this amino acid but are also methods for phenolic compounds in general and the possibility that the tyrosine figures may include values for non-tyrosine phenolic compounds cannot be ignored. Nevertheless, chromatograms of all hydrolysed residues, both of rheumatic and non-rheumatic, suggest that there is indeed more tyrosine than in tendon, gelatin and elastin. Although the tyrosine content of non-rheumatic residue is comparable in amount with that of rheumatic residue, the absolute quantity in the nodules is much higher than in non-rheumatic tissue, since the residue of the former is much greater. Similar conclusions apply to the carbohydrate constituents. In both rheumatic and non-rheumatic tissues, the sugars released on hydrolysis are the same, and in both cases they appear to occur in similar relative proportions to each other. This suggests that the polysaccharide components normally present in subcutaneous tissue occur also in rheumatic lesions. There is little reason for supposing that the nodules contain substances which are not normally present, but the results suggest that the non-collagen protein and polysaccharide occur locally in higher amounts in the nodules. This may be due either to increased deposition of these substances or to destruction and removal of collagen.

The analytical results of non-rheumatic tissue itself call for some comment. The existence of acidic mucopolysaccharides in the interfibrillary substance of the mesenchyme has long been postulated, and both hyaluronic acid and chondroitin sulphate have been isolated from animal skin by Meyer & Chaffee, 1941 (cf. Rienits, 1953), and from

human skin by Pearce & Watson (1949). The absence of uronic acid and galactosamine in the mild hydrolysates of the tissue, whilst not evidence for the absence of hyaluronic acid and chondroitin sulphate, in view of the small quantities used for analysis, does indicate that, if present in subcutaneous tissue, the amounts of these polysaccharides are small compared with that of other uronic-acid-free polysaccharides. Further, if hyaluronic acid were present in sufficient amount, mild hydrolysis would have also given hyalobiuronic acid (Rapport, Weissmann, Linker & Meyer, 1951), which has always been observed in hydrolysates of hyaluronic acid preparations (Consdén, 1953*b*). The presence of galactose in the hydrolysates of tissue, apparently as a major sugar constituent, suggests the presence of a galactose-rich polysaccharide. Some of this material, particularly in the residues, would appear to form part of the interfibrillary ground substance, especially since Little & Kramer (1952) have shown that this is resistant to boiling water. Few chemical studies have been carried out on this material, although it is generally accepted that it is mucoprotein in nature. Our high tyrosine values in the residue are in line with the work of Lisco (1949), who found evidence for the presence of protein, rich in aromatic amino acid, in beef and rat-tail tendons, but it is evident that further chemical work on the non-collagen, non-elastin constituents of normal subcutaneous tissue itself is necessary before one can obtain a clearer picture of the changes in pathological tissue.

SUMMARY

1. Subcutaneous elbow nodules from rheumatic-fever patients have been fractionated by autoclaving into collagen-rich extracts and non-collagen residues. Qualitative and quantitative analysis of the hydrolysed fractions were carried out and compared with analyses of non-rheumatic subcutaneous elbow tissue treated similarly.

2. In addition to collagen and elastin, the nodules contained a tyrosine-rich substance and polysaccharide. These appear to be present in non-rheumatic tissue, but in much smaller amount. The presence of large quantities of fibrin in nodules is regarded as improbable.

3. The predominant polysaccharide constituents of both non-rheumatic and rheumatic subcutaneous tissues are rich in galactose and glucosamine.

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REFERENCES

- Bien, E. G. & Ziff, M. (1951). *Proc. Soc. exp. Biol.*, N.Y., **78**, 327.
- Bowes, J. H. (1951). *Research, Lond.*, **4**, 155.
- Bowes, J. H. & Kenten, R. H. (1949). *Biochem. J.* **45**, 281.
- Consdén, R. (1953*a*). *Disc. Faraday Soc.* **13**, 282.
- Consdén, R. (1953*b*). *Disc. Faraday Soc.* **13**, 286.
- Consdén, R., Glynn, L. E. & Stanier, W. M. (1952). *Biochem. J.* **50**, xix.
- Consdén, R. & Gordon, A. H. (1950). *Biochem. J.* **46**, 8.
- Consdén, R., Gordon, A. H. & Martin, A. J. P. (1944). *Biochem. J.* **38**, 224.
- Consdén, R. & Stanier, W. M. (1952*a*). *Nature, Lond.*, **169**, 783.
- Consdén, R. & Stanier, W. M. (1952*b*). *Nature, Lond.*, **170**, 1069.
- Elson, L. E. & Morgan, W. T. J. (1933). *Biochem. J.* **27**, 1824.
- Folin, O. & Ciocalteu, V. (1927). *J. biol. Chem.* **73**, 627.
- Glynn, L. E. & Loewi, G. (1952). *J. Path. Bact.* **64**, 329.
- Gomöri, G. (1937). *Amer. J. Path.* **13**, 993.
- Goodwin, T. W. & Morton, R. A. (1946). *Biochem. J.* **40**, 628.
- Hall, D. A. (1951). *Nature, Lond.*, **168**, 513.
- Holiday, E. R. (1936). *Biochem. J.* **30**, 1795.
- Johns, R. G. S. & Marrack, J. R. (1952). *Biochem. J.* **50**, xvii.
- Johnston, J. P., Ogston, A. G. & Stanier, J. E. (1951). *Analyst*, **76**, 899.
- Kellgren, J. H., Ball, J., Astbury, W. T., Reed, R. & Beighton, E. (1951). *Nature, Lond.*, **168**, 493.
- Lisco, H. (1949). *Arch. Biochem.* **22**, 406.
- Little, K. & Kramer, H. (1952). *Nature, Lond.*, **170**, 499.
- Meyer, K. & Chaffee, E. (1941). *J. biol. Chem.* **138**, 491.
- Neuman, R. E. (1949). *Arch. Biochem.* **24**, 289.
- Neuman, R. E. & Logan, M. A. (1950*a*). *J. biol. Chem.* **184**, 299.
- Neuman, R. E. & Logan, M. A. (1950*b*). *J. biol. Chem.* **186**, 549.
- Partridge, S. M. (1948). *Biochem. J.* **42**, 238.
- Partridge, S. M. (1949). *Nature, Lond.*, **164**, 443.
- Pearce, R. H. & Watson, E. M. (1949). *Canad. J. Res.* **27E**, 43.
- Rapport, M. M., Weissmann, B., Linker, A. & Meyer, K. (1951). *Nature, Lond.*, **168**, 996.
- Rienits, K. G. (1953). *Biochem. J.* **53**, 79.
- Shaffer, P. A. & Somogyi, M. (1933). *J. biol. Chem.* **100**, 695.
- Spies, J. R. & Chambers, D. C. (1948). *Analyt. Chem.* **20**, 30.
- Tristram, G. R. (1949). *Advanc. Protein Chem.* **5**, 84.

Studies on Sulphatases

3. THE ARYLSULPHATASE AND β -GLUCURONIDASE OF MARINE MOLLUSCS

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Arylsulphatase was first demonstrated in molluscs by Derrien (1911) who found that extracts of a large marine gastropod, *Murex trunculus*, could hydrolyse indoxyl sulphate. Soda & Egami (1933) and Soda (1936) studied the distribution of the enzyme in a large number of tropical marine molluscs common to Japanese waters. Considerable activity was observed amongst members of the Gastropoda, Pelecypoda, Cephalopoda and Amphineura. It was also found in some Arthropoda. In all cases the enzyme activity was mainly concentrated in the digestive glands. Information regarding the properties of the mollusc enzyme is scanty. Soda (1936) recorded an optimum pH of 7 for the arylsulphatase of *Charonia lampas* (*Tritonium nodiferum*) when potassium phenylsulphate was the substrate, although Soda & Egami (1941) used pH 5.2 for enzyme assay. The substrate concentration used by these workers (0.05M) was apparently arbitrary and not necessarily optimal. The high arylsulphatase activity of extracts of the digestive glands of

Haliotis gigantea (Soda & Koyama, 1935) was confirmed by Morimoto (1937) using potassium *p*-nitrophenylsulphate as substrate under arbitrary experimental conditions. Tanaka (1938) found that the maximum hydrolysis of potassium *p*-nitrophenylsulphate by aqueous extracts of digestive glands of various marine organisms occurred at pH 5.0–5.2 when the substrate concentration was 0.0005M. The optimum substrate concentration was not recorded.

During preliminary studies (Dodgson, Lewis & Spencer, 1952) the digestive organs of many marine molluscs common to British waters were found to be rich in arylsulphatase and promising as starting material for the preparation of enzyme concentrates. It was noted that the organisms also possessed marked β -glucuronidase activity. Nagaoka (1951) used the β -glucuronidase of certain terrestrial snails, but there appears to be no previous record of the occurrence of this enzyme in marine molluscs. In view of the desirability of