STATUTORY INSTRUMENTS

# 1966 No. 501

# THERAPEUTIC SUBSTANCES

## The Therapeutic Substances (Manufacture of Enzymes and Enzyme Inhibitors) Regulations 1966

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| *Made - - -* | *27th April* 1966 |
| *Laid before Parliament* | *5th May* 1966 |
| *Coming into Operation* | *1st June* 1966 |

The Joint Committee constituted by section 4(1) of the Therapeutic Substances Act 1956(a), in exercise of the powers conferred on them by sections 1 and 5 of the said Act, after consultation with the Advisory Committee constituted under section 4(2) of the said Act, hereby make the following regulations:—

### PART I

### PRELIMINARY

*Citation, Commencement and Interpretation*

**1.**—(1) These regulations may be cited as the Therapeutic Substances (Manufacture of Enzymes and Enzyme Inhibitors) Regulations 1966 and shall come into operation on 1st June 1966.

(2) In these regulations, unless the context otherwise requires—

" the Act " means the Therapeutic Substances Act 1956;

" the general regulations " means the Therapeutic Substances (Manufacture and Importation) General Regulations 1963, as amended (b);

" the licensing authority " has the same meaning as in the general regulations;

" parenteral injection " in relation to any substance includes any administration of the substance by insertion into the body by any route other than that of the gastro-intestinal tract but does not include any application of the substance to the skin.

(3) Any reference in these regulations to any provision of the general regulations shall be construed as a reference to that provision as amended, extended or re-enacted by any subsequent regulations made under part I of the Act.

(4) For the purposes of these regulations the cubic centimetre may be used wherever the millilitre is indicated.

(5) The Interpretation Act 1889(c) shall apply to the interpretation of these regulations as it applies to the interpretation of an Act of Parliament.

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(a) 4 & 5 Eliz. 2. c. 25.
(b) S.I. 1963/1450, 1964/1076 (1963 II, p. 2483; 1964 II, p. 2380).
(c) 52 & 53 Vict. c. 63.

*Revocation of existing regulations and inclusion of substances in schedule to Act*

**2.**—(1) The Therapeutic Substances (Manufacture of Enzymes and Enzyme Inhibitors) Regulations 1963(**a**) and the Therapeutic Substances (Manufacture of Enzymes and Enzyme Inhibitors) Amendment Regulations 1964(**b**) are hereby revoked.

(2) Notwithstanding the provisions of paragraph (1) of this regulation, the enzymes and enzyme inhibitors to which parts III to VIII of these regulations relate, and preparations of any of them, (other than hyaluronidase and heparin, in each case not being intended for parenteral injection, and preparations thereof,) shall continue to be included in schedule 1 to the Act.

## PART II

### GENERAL PROVISIONS RELATING TO ENZYMES AND ENZYME INHIBITORS AND TO PREPARATIONS THEREOF

*Tests for sterility*

**3.** Enzymes and enzyme inhibitors to which the succeeding parts of these regulations apply, and preparations of any of them, shall be tested for sterility in accordance with the general regulations.

*Labelling*

**4.**—(1) Subject to the provisions of paragraph (2) of this regulation—

*(a)* the particulars specified in regulation 6(1), (a), (b) and (c) of the general regulations shall be borne by the container of any substance to which these regulations relate;

*(b)* the particulars specified in sub-paragraph (g) of the said regulation 6(1) shall be borne both by the container of any such substance and by the package in which it is offered for sale;

*(c)* the particulars specified in sub-paragraphs (e), (f), (h) and (i) of the said regulation 6(1) shall be borne either by the container of any such substance or by the package in which it is offered for sale.

(2) Where by any provision of these regulations any particulars are required to be borne by the container of any substance to which these regulations relate and such container is of a capacity of less than 2·5 millilitres, such particulars may, with the consent of the licensing authority, be borne instead by the package in which the container is offered for sale.

## PART III

### SPECIAL PROVISIONS RELATING TO HYALURONIDASE

*Definition and proper name*

**5.** Hyaluronidase is a preparation, or derivative, prepared from mammalian testicles or sperm or from other sources approved by the licensing authority, of the enzymes which diminish the viscosity of the hyaluronic acid present in mammalian tissues. Its proper name is " hyaluronidase ".

*Standard preparation*

**6.** The standard preparation is one of dried hyaluronidase kept at the National Institute for Medical Research, London.

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(**a**) S.I. 1963/1454 (1963 II, p. 2521).
(**b**) S.I. 1964/1431 (1964 III, p. 3310).

*Unit of standardisation*

**7.** The unit of hyaluronidase for the purpose of these regulations is the activity contained in such an amount of the standard preparation as is for the time being indicated by the licensing authority, on the advice of the Medical Research Council, as the quantity exactly equivalent to the unit accepted for international use.

*Test for potency*

**8.**—(1) Hyaluronidase shall be tested for potency by comparative tests in relation to the standard preparation by a method approved by the licensing authority.

(2) The potency so determined shall be expressed in units per milligram of the solid form, and in units per millilitre of solutions.

(3) Hyaluronidase in the solid form with a potency of less than 300 units per milligram calculated with reference to the dried substance shall not be issued.

*Test for freedom from abnormal toxicity*

**9.**—(1) Hyaluronidase shall be tested for freedom from abnormal toxicity in accordance with this regulation.

(2) A quantity of hyaluronidase containing not less than 2,500 units shall be made up in a volume of physiological saline not exceeding 0·25 millilitre and shall be injected subcutaneously into each of 5 healthy mice each weighing between 17 and 22 grams. The sample shall pass the test if either:—

*(a)* the injection does not cause death or necrosis of the skin in any of the mice within 48 hours; or

*(b)* the injection having caused death or necrosis of the skin in one only of the mice within that period, similar injections in 5 other such mice do not cause the death or necrosis of the skin of any of those mice within 48 hours.

*Freedom from pyrogenic substances*

**10.**—(1) Hyaluronidase shall be free from pyrogenic substances to such an extent that, if tested for freedom from any such substances in accordance with the general regulations, it would pass the test.

(2) The number of units of hyaluronidase for the purpose of the said test shall not be less than 250 units per kilogram of the weight of each rabbit used in the test.

*Quality*

**11.** Hyaluronidase in solution, and the dry solid when reconstituted with freshly boiled and cooled water so as to give a concentration of 3 milligrams per millilitre, shall have the following characteristics:—

*(a)* the solution shall be a clear colourless or faintly yellow liquid;

*(b)* the solution shall have a pH value between 4·5 and 7·5.

*Method of issue*

**12.** Hyaluronidase shall be issued either in a watery solution or as a dry solid for reconstitution with a watery solvent.

*Labelling*

**13.**—(1) In addition to the particulars required by paragraph (1)*(a)* and *(b)* of regulation 4 of these regulations the following particulars shall, except as provided in paragraph (2) of the said regulation 4, be borne by a container of hyaluronidase:—

*(a)* in the case of a solid preparation, the total number of international units in the container;

*(b)* in the case of a solution, the number of international units per millilitre of the solution;

*(c)* in either case:—

(i) a statement that the preparation is not intended for intravascular injection; and

(ii) a statement of the species of animal from which the substance is derived.

(2) In the case of a preparation of hyaluronidase in solution, in addition to the particulars required by paragraph (1)*(c)* of regulation 4 of these regulations, particulars of the nature of the solvent and of any stabilising agent shall, except as provided in paragraph (2) of the said regulation 4, be borne either by the container of the solution or by the package in which the solution is offered for sale.

## PART IV

### SPECIAL PROVISIONS RELATING TO STREPTOKINASE

*Definition and proper name*

**14.** Streptokinase is the substance produced by certain strains of streptococcus which activates an inactive precursor of a fibrinolytic enzyme present in human plasma. Its proper name is " streptokinase ".

*Method of preparation*

**15.** Streptokinase shall be prepared, by methods approved by the licensing authority, from strains of streptococci other than those belonging to Lancefield's Group A.

*Standard preparation*

**16.** The standard preparation in relation to streptokinase is one of streptokinase-streptodornase kept at the National Institute for Medical Research, London.

*Unit of standardisation*

**17.** The unit of streptokinase for the purpose of these regulations is the specific activity contained in such an amount of the standard preparation of streptokinase-streptodornase as is for the time being indicated by the licensing authority, on the advice of the Medical Research Council, in relation to streptokinase.

*Quality*

**18.**—(1) Streptokinase, when dissolved according to the manufacturer's directions, or, if there are no such directions, in physiological saline, shall have a pH value lying between 6·0 and 8·5.

(2) Streptokinase shall not contain more than 100 HD50 of streptolysin for each 100,000 units of streptokinase. For this purpose an HD50 of streptolysin is that amount of streptolysin in the reduced form which will cause 50 per cent. haemolysis of 5·0 millilitres of a 0·7 per cent. suspension of fresh washed red cells of a rabbit at 37°C. in 30 minutes.

*Test for potency*

**19.** Streptokinase shall be tested for potency by measurement of its ability to lyse a preformed clot of fibrinogen containing human plasminogen, by comparison against the standard preparation mentioned in regulation 16 of these regulations, and such potency shall be expressed in terms of the unit of standardisation as defined in regulation 17 of these regulations.

*Test for freedom from abnormal toxicity*

**20.**—(1) Streptokinase shall be tested for freedom from abnormal toxicity in accordance with this regulation.

(2) A quantity of streptokinase containing 10,000 units of streptokinase, in a volume not exceeding 5·0 millilitres of a watery solution, shall be injected intravenously into a healthy rabbit weighing between 2 and 2·5 kilograms and subcutaneously into each of 3 healthy guinea pigs each weighing between 250 and 350 grams which have not been previously treated with any material which will interfere with the test; and a quantity thereof containing 1,000 units of streptokinase, in a volume not exceeding 0·5 millilitre of a watery solution, shall be injected intraperitoneally into each of 3 healthy mice each weighing between 17 and 22 grams. The sample shall pass the test if all the animals survive for 7 days without showing significant signs referable to the injected substance, or if one of the guinea pigs or one of the mice dies within this period, further such injections into 3 other such guinea pigs or 3 other such mice do not result in the death of, or in the appearance of significant signs referable to the injected substance in, any of them within 7 days following such further injections.

*Labelling*

**21.** In addition to the particulars required by paragraph (1)*(a)* and *(b)* of regulation 4 of these regulations, the following further particulars shall, except as provided in paragraph (2) of the said regulation 4, be borne by the container of streptokinase:—

*(a)* the number of units of streptokinase contained in the container;

*(b)* the nature of the buffer, if any, contained in the preparation;

*(c)* a statement that the preparation is not intended for intravascular injection.

## PART V

### SPECIAL PROVISIONS RELATING TO PREPARATIONS OF STREPTOKINASE INTENDED FOR INTRAVASCULAR ADMINISTRATION

*Method of preparation*

**22.** Preparations of streptokinase intended for intravascular administration shall be prepared from streptokinase complying with Part IV of these regulations.

*Quality*

**23.** Preparations of streptokinase intended for intravascular administration—

*(a)* when dissolved according to the manufacturer's directions, or, if there are no such directions, in physiological saline, shall have a pH value lying between 7·0 and 7·5;

*(b)* shall not contain more than 5 HD50 of streptolysin for each 100,000 units of streptokinase, and for this purpose an HD50 of streptolysin shall be the amount specified in regulation 18(2) of these regulations;

*(c)* shall not contain more than 10 units of streptodornase for each 100,000 units of streptokinase when estimated by a method approved by the licensing authority.

*Potency*

**24.** Preparations of streptokinase intended for intravascular administration—

*(a)* shall be tested for potency in their final form by measurement of their ability to lyse a preformed clot of fibrinogen containing human plasminogen, by comparison against the standard preparation mentioned in regulation 16 of these regulations, and such potency shall be expressed in terms of the unit of standardisation as defined in regulation 17 of these regulations;

*(b)* shall have a potency of not less than 300 units per microgram of protein nitrogen contained in each preparation.

*Test for freedom from pyrogenic substances and from abnormal toxicity*

**25.**—(1) Preparations of streptokinase intended for intravascular administration shall be tested for freedom from pyrogenic substances in accordance with the general regulations, and the same test shall be used as a test for freedom from abnormal toxicity.

(2) The quantity of any such preparation for the purpose of any such test shall be 20,000 units per kilogram of the weight of each rabbit used in the test.

(3) The preparation shall be regarded as free from abnormal toxicity if all the rabbits used in the test survive for 7 days without showing significant signs referable to the injected substance.

*Labelling*

**26.**—(1) In addition to the particulars required by paragraph (1)*(a)* and *(b)* of regulation 4 of these regulations, the following further particulars shall, except as provided in paragraph (2) of the said regulation 4, be borne by the container of a preparation of streptokinase intended for intravascular administration:—

*(a)* in the case of a solid preparation, the minimum number of units per milligram, and the total number of units of streptokinase in the container;

*(b)* in the case of a solution, the number of units per millilitre, and the total number of units of streptokinase in the container;

*(c)* the nature of the buffer and the diluent (if any) in the container;

*(d)* the nature of the vehicle recommended by the manufacturer for dissolving or diluting the preparation;

*(e)* a statement that the preparation is intended for intravascular administration;

*(f)* the maximum period of time after dissolving the preparation after which the solution must not be administered.

(2) In addition to the particulars required by paragraph (1)*(c)* of regulation 4 of these regulations, the following further particulars shall, except as provided in paragraph (2) of the said regulation 4, be borne either by the container of

a preparation of streptokinase intended for intravascular administration or by the package in which it is offered for sale:—

*(a)* the method of administration recommended;

*(b)* a statement that the material is antigenic.

## PART VI

### SPECIAL PROVISIONS RELATING TO STREPTODORNASE

*Definition and proper name*

**27.** Streptodornase is the substance produced by certain strains of streptococcus which catalyzes the depolymerisation of deoxyribonucleic acid. Its proper name is " streptodornase ".

*Application of part IV*

**28.** Regulations 15 to 21 of these regulations shall apply to streptodornase with the substitution for references to streptokinase (except in the expression " streptokinase-streptodornase ") of references to streptodornase.

## PART VII

### SPECIAL PROVISIONS RELATING TO STREPTOKINASE-STREPTODORNASE

*Definition and proper name*

**29.** Streptokinase-streptodornase is a combination of streptokinase and streptodornase prepared by the growth of streptococci in a suitable liquid medium and subsequently purified therefrom. Its proper name is " streptokinase-streptodornase ".

*Application of part IV*

**30.** Regulations 15 and 17 and regulation 18(1) of these regulations shall apply to streptokinase-streptodornase with the substitution for references to streptokinase of references to streptokinase-streptodornase.

*Quality*

**31.** Streptokinase-streptodornase shall not contain more than 100 HD50 of streptolysin for each 100,000 units of streptokinase or for each 100,000 units of streptodornase, whichever has the greater number of units in the preparation being tested. For this purpose an HD50 of streptolysin is that amount of streptolysin in the reduced form which will cause 50 per cent. haemolysis of 5·0 millilitres of a 0·7 per cent. suspension of fresh washed red cells of a rabbit at 37°C. in 30 minutes.

*Test for potency*

**32.** Streptokinase-streptodornase shall be tested for potency of both constituents by comparative tests in relation to the standard preparation by methods approved by the licensing authority.

*Test for freedom from abnormal toxicity*

**33.**—(1) Streptokinase-streptodornase shall be tested for freedom from abnormal toxicity in accordance with this regulation.

(2) A quantity of streptokinase-streptodornase containing 10,000 units of streptokinase or 10,000 units of streptodornase, in a volume not exceeding 5·0 millilitres of a watery solution, shall be injected intravenously into a healthy rabbit weighing between 2 and 2·5 kilograms and subcutaneously into each of

3 healthy guinea pigs each weighing between 250 and 350 grams which have not been previously treated with any material which will interfere with the test; and a quantity thereof containing 1,000 units of streptokinase or 1,000 units of streptodornase in a volume not exceeding 0·5 millilitre of a watery solution shall be injected intraperitoneally into each of 3 healthy mice each weighing between 17 and 22 grams. The sample shall pass the test if all the animals survive for 7 days without showing significant signs referable to the injected substances, or if one of the guinea pigs or one of the mice dies within this period, further such injections into 3 other such guinea pigs or 3 other such mice do not result in the death of, or in the appearance of significant signs referable to the injected substance in, any of them within 7 days following such further injections.

*Labelling*

**34.** In addition to the particulars required by paragraph (1)*(a)* and *(b)* of regulation 4 of these regulations the following further particulars shall, except as provided in paragraph (2) of the said regulation 4, be borne by the container of streptokinase-streptodornase:—

*(a)* the number of units of streptokinase and the minimum number of units of streptodornase contained in the container;

*(b)* the nature of the buffer, if any, contained in the preparation;

*(c)* a statement that the preparation is not intended for intravascular injection.

## PART VIII

### SPECIAL PROVISIONS RELATING TO HEPARIN AND PREPARATIONS THEREOF

*Definitions and proper names*

**35.**—(1) For the purposes of these regulations, heparin is a substance containing the sodium salt of a sulphated polysaccharide obtained from mammalian tissue, which has the property of prolonging the clotting-time of blood in man or other animals.

(2) The proper name for heparin obtained from mammalian lung is " heparin (lung) " and the proper name for heparin obtained from mammalian intestinal mucosa is " heparin (mucous) ". The proper name for a preparation of either kind of heparin is " heparin (lung) " or " heparin (mucous) ", as may be appropriate, qualified by such word or words as may be approved by the licensing authority as indicating the nature of the preparation.

*Standard preparations*

**36.** The standard preparations of heparin (lung) and of heparin (mucous) are ones kept at the National Institute for Medical Research, London.

*Unit of standardisation*

**37.** The unit of heparin (lung) or of heparin (mucous) for the purpose of these regulations is the specific activity contained in such an amount of the appropriate standard preparation as is for the time being indicated by the licensing authority, on the advice of the Medical Research Council, as the quantity exactly equivalent to the unit accepted for international use.

*Quality*

**38.** Heparin (lung) or heparin (mucous) shall have the following characteristics:—

*(a)* it shall be in the form of a white or creamy-white amorphous powder which is odourless or nearly so, and hygroscopic;

*(b)* it shall be soluble, at 20°C., in not more than 2·5 parts of physiological saline, and in a 1 per cent. w/v solution in physiological saline shall have a pH value lying between 6·0 and 8·0;

*(c)* it shall have a specific optical rotation of +35° to +55° when determined on a 4 per cent. w/v watery solution at 20°C.;

*(d)* on drying to constant weight over phosphorus pentoxide at 60°C. at a pressure not exceeding 5 millimetres of mercury, it shall not lose more than 12 per cent. of its weight;

*(e)* when 0·25 millilitre of a 5 per cent. w/v solution of trichloroacetic acid is added to 1 millilitre of a 1 per cent. watery solution of heparin no turbidity or precipitation shall occur;

*(f)* it shall contain, in the case of heparin (lung), not less than 110 units per milligram, and in the case of heparin (mucous), not less than 130 units per milligram, calculated in each case with reference to the material dried to constant weight at 60°C. at a pressure not exceeding 5 millimetres of mercury.

*Tests for potency*

**39.** Heparin (lung) and heparin (mucous) and preparations thereof shall be tested biologically for potency by a method approved by the licensing authority by comparison with the appropriate standard preparation, and the potency and the fiducial limits of error (P=0·95) of the potency shall be calculated from the results of the test. The estimated potency shall be not greater than 111 per cent. or less than 90 per cent. of the potency stated by the manufacturer. The percentage fiducial limits of error (P=0·95) of the estimated potency shall lie between 80 per cent. and 125 per cent. of the potency stated by the manufacturer.

*Freedom from depressor substances*

**40.**—(1) Heparin (lung) and heparin (mucous) shall, if the licensing authority so require, be tested for freedom from depressor substances, and in any event shall be free from such substances to such an extent that, if tested for freedom from such substances in accordance with this regulation, they would pass the test.

(2) A quantity of heparin (lung) or of heparin (mucous) containing not less than 1,000 units of heparin (lung) or of heparin (mucous) dissolved in one millilitre of water or of physiological saline shall be injected intravenously into a cat anaesthetised with chloralose or a suitable barbiturate. The sample shall pass the test if the injection causes a smaller fall of arterial blood pressure than that produced by the injection into the same cat of a solution of histamine salt containing 0·3 microgram of histamine base in one millilitre of water or of physiological saline.

*Freedom from pyrogenic substances*

**41.**—(1) Heparin (lung) and heparin (mucous) shall, if the licensing authority so require, be tested for freedom from pyrogenic substances, and in any event shall be free from such substances to such an extent that, if tested for freedom from such substances in accordance with the general regulations, they would pass the test.

(2) The quantity of heparin (lung) or of heparin (mucous) for the purpose of the said test shall be, in either case, 500 units per kilogram of the weight of each rabbit used in the test.

*Labelling*

**42.** In addition to the particulars required by paragraph (1)*(a)* and *(b)* of regulation 4 of these regulations, the following further particulars shall, except as provided in paragraph (2) of the said regulation 4, be borne by a container of heparin (lung) or of heparin (mucous) or of any preparation thereof:—

*(a)* the number of units of heparin contained in the container;

*(b)* in the case of a solid preparation, the number of units per milligram of the contents;

*(c)* in the case of a solution or suspension, the number of units per millilitre thereof.

These regulations were made by the aforesaid Joint Committee on 27th April 1966.

*Kenneth Robinson,*
*William Ross,*
*William J. Morgan,*
Members of the Joint Committee

*Wilfred Turner,*
Clerk to the Joint Committee

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## EXPLANATORY NOTE

*(This Note is not part of the Regulations.)*

These regulations made under Part I of the Therapeutic Substances Act 1956 consolidate, with amendments, the Therapeutic Substances (Manufacture of Enzymes and Enzyme Inhibitors) Regulations 1963 and 1964.

The regulations apply to the following enzymes and enzyme inhibitors, namely, hyaluronidase, streptokinase, streptokinase-streptodornase and heparin, and preparations of any of them, and in particular provide for:—

*(a)* standards of quality;

*(b)* tests for sterility, potency, freedom from abnormal toxicity and other tests appropriate to the particular substance; and

*(c)* requirements as to labelling.