

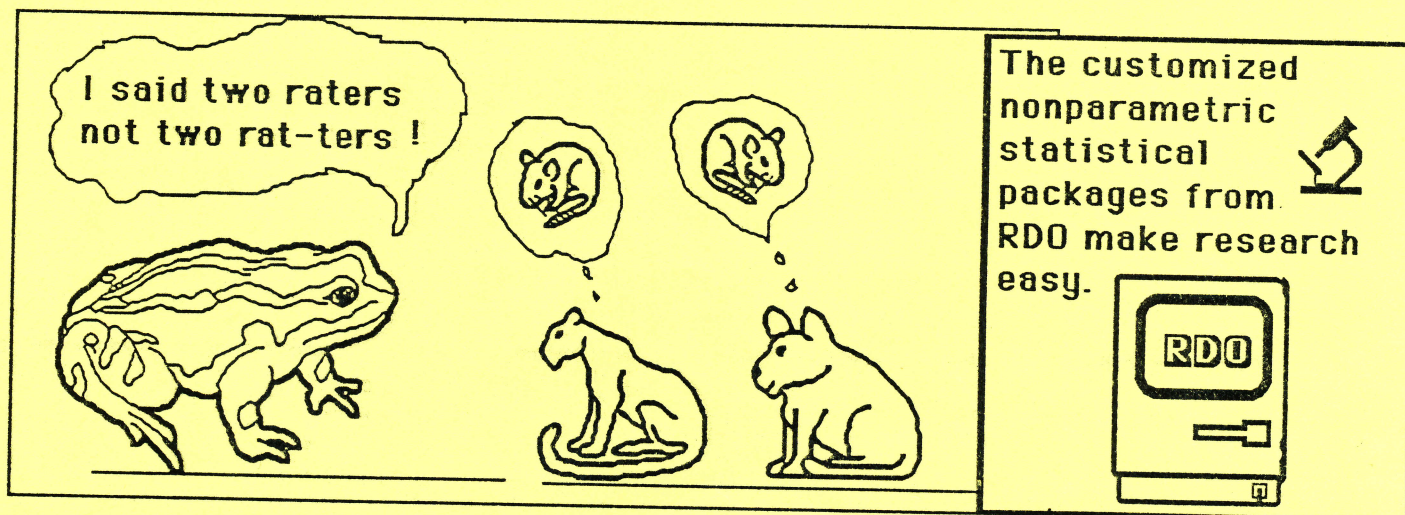
Dental OCTOBER 1990 Research News

Research Development Office, (902) 494-1675

VOLUME IV, NUMBER 10.

Let Your Research 'EXCEL'
The Research Development Office is pleased to announce that two further nonparametric statistical packages have been programmed using Microsoft Excel. This is in addition to the RIDIT ANALYSIS which was reported in last months Dental Research News. The new programmes are a test for **Significance Between Two Proportions** and a **Measurement of Inter rater Agreement** (the Cohen-Kappa test). The availability of these three nonparametric

analysis systems which can be customized for individual requirements addresses a very real need for a large number of research projects being conducted within our faculty. In all three cases the programmes can be provided on individual discs which simply require the raw data to be input, no knowledge of the statistical procedure is required. If you think that you may be able to use any of these statistical programmes contact the Research Development Office for further details.



Against All Odds

The explanation for the origin of life on Earth depends on the extraordinary probability that a group of amino acids would randomly come together to form a single protein. This probability has in the past been estimated at odds of 10^{131} to one. Some new calculations by Dr's Ken Dill and Kit Lau of the University of California at San Francisco, have significantly reduced these odds.

The molecular chains of amino acids which form proteins, bring about the chemical reactions that are essential to life. The accepted hypothesis of the beginning of life on Earth states that billions of years ago a group of amino acids floating around in a primordial ocean, just happened to be in the right place at the right time to join together, in the proper order, to form a protein. However, the high statistical probability against this happening has long bothered researchers. For a random soup of amino acids and amino acid chains, to produce something that's protein-like and has biological activity would seem to be a real long shot.

The possibility of this happening would seem to be extremely unlikely. A typical small protein has 100 amino acids. There are some twenty different naturally occurring

amino acids. If you raise 20 to the hundredth power (the number of possible amino acid positions in a protein), you get 10 followed by 130 zeros. You would have to make that many chains of 100 amino acids to be sure of getting any particular protein.

Fortunately, a protein must fold in certain ways to bring about chemical reactions, and a variety of amino sequences can fold to cause the same reaction. However, the task of finding the elaborate folding patterns of every possible amino sequence has in the past prevented biologists from taking the folding into account.

The researchers at University of California at San Francisco have now created the first computer program that can translate an amino sequence into a simple model of its folding pattern. Dill and Lau have found that one in 10 billion chains of 100 amino acids will fold into a biologically useful form. This is some 120 fewer zeros than in the original calculation of the odds of life beginning. This means that the chances of random combinations of amino acids forming proteins are now very significantly greater than researchers had thought in the past.

WHMIS Problem for 38%?

As many as four in 10 Canadian workers will have trouble understanding a new information system about hazardous materials in the work-place. A literacy expert professor Stan Jones of Carleton University has said that some of the literature in the national system is likely beyond the reading ability of 38 percent of Canadians.

About 6.8 million people, have trouble with everyday reading tasks, according to a Statistics Canada survey released in May 1990.

The Work-place Hazardous Materials Information System, (WHMIS) in effect at Dalhousie University in our laboratories and clinic areas as well as across Canada, is designed to ensure that employers and employees have full information about the safe handling of hazardous products.

The system is a co-ordinated set of laws passed at the federal and provincial level and has been called the most far-reaching health-and-safety legislation ever passed in Canada.

It requires the employer to label hazardous products, train staff, and make available information sheets that describe each material's properties and how first aid should be administered.

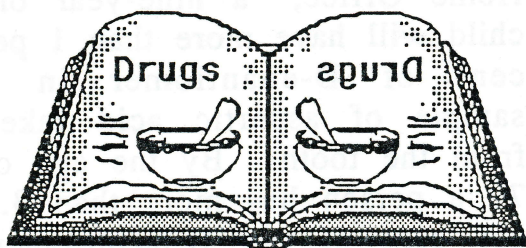
It covers the overwhelming majority of workers in the country.

Professor Stan Jones, who was a consultant on the Statistics Canada study, said he has concerns about the design of the labels and the way information is presented in the data sheets. The sheets are produced by manufacturers and vary in length, detail, format and simplicity of language. He wonders whether any attention was paid to the readability of the symbols and written warnings when the information system was being put together.

Morley Brownstein, federal co-ordinator of the system for Consumer and Corporate Affairs, said it's misleading to say four in 10 workers can't use the system. He said it's important to remember the system consists of detailed training as well as data sheets, and that it makes use of well-known symbols, thus avoiding words altogether. The data sheets, kept in the work-place, tell employees how to handle an emergency, like spillage or ingestion of a hazardous product. Brownstein said the government didn't standardize the data sheets in a simple format because "what we were trying to do is not have any more regulation than is absolutely necessary."

Mirror Image Drugs

Commonly prescribed drugs consist of chiral mixtures: pairs of enantiomeric molecules which are mirror images of each other. The two enantiomers often have different pharmacological properties. At best the wrong enantiomer being inactive, and at worst giving rise to side-effects or in some cases even antagonising the desired drug activity. Researchers have been working for some years on ways of making single enantiomers. The use of micro-organisms in the drug synthesis, known as bio-transformation, is now proving successful. Recently, single enantiomers of Ibuprofen (Advil, Profen) a non-steroidal anti-inflammatory drug was reported by a scientist from the Merck, Sharp and Dohm drug company



Commitment to Science

The 100th anniversary of *The Merck index* has been marked by the publication of its 11th edition. Some 60 per cent of this well known reference book has been completely revised since the 10th edition in 1983.

New entries include Aids and cancer drugs, novel natural products, genetically engineered drugs and herbicides, insecticides, pesticides and suspected carcinogens. Other new features are a therapeutic category and a biological activity index.

The Merck index has come a long way from the 1889 edition, which was a simple catalogue of chemicals produced by Merck. It is a convenient one-volume quick reference to a large number of drugs and chemicals, is used in laboratories world-wide, and is also available on line. *The Merck index* is published on a non-profit making basis as part of Merck's commitment to the advancement of science. *The Merck index*, 11th edition, S. Budavari (ed), is published by Merck, New Jersey, 1989, ISBN 91191028X at \$35.00.



SMILE

Research Definition

"It is clear that much additional work will be required before a complete understanding of the phenomenon is possible."

means:-

I don't understand it.

An Age—Old Problem.

When a forensic dentist is summoned to the scene of a crime committed years ago, one of the most important things he or she has to tell police is the age of the body. The police can then narrow down their search through the missing person files to try to identify the body. If the victim was aged 22 or less, then the job is much easier since teeth develop sequentially and reliably, and the forensic dentist simply has to look at the number of teeth that have erupted. But after 22, the task is not so straightforward and more searching procedures have to be adopted.

Previously, the standard method used by forensic dentists to estimate the age of older bodies was to measure the translucency of the teeth. The mineral content of teeth changes throughout a person's life. The new mineral deposited has a different refractive index to that which is deposited at birth, and dentists have taken advantage this fact to determine the age of a deceased individual to the nearest 10 years.

Three or four years ago, researchers in the chemistry department of the University of Wales College of Cardiff and the dental school of the University of Wales College of Medicine

(UWCM) in Cardiff, got together to try to improve on this accuracy. They decided to make use of the enantiomeric properties of aspartic acid in teeth to determine the age of individuals at the time of death.

Their research was based upon a discovery, made in the US some 10 years ago, that the dentine in teeth contains aspartic acid, which is locked into collagen as the tooth forms and is hardened through the deposition of several layers of the mineral calcium hydroxyapatite ($\text{Ca}_5(\text{OH})(\text{PO}_4)_3$). The initial aspartic acid deposit is the L-enantiomer of the chiral molecule. Over the years, racemisation occurs and the D-enantiomer begins to build up. According to Dr. David Whittaker, reader in oral biology at UWCM and forensic dentistry adviser to the UK Home Office, "a nine-year old child will have more than 1 per cent of D-enantiomer in a sample of aspartic acid taken from the tooth. By the age of 70 a person has more than 3.3 per cent of the D-enantiomer. The difference is not great, but it is reliable."

A five step process following tooth extraction has been developed by the researchers

- 1) The tooth is dissolved in hydrochloric acid to remove the mineral content, leaving the collagen. (Cont on Page 6)

An Age—Old Problem.

(Cont from page 5)

2) The collagen is then partially hydrolysed by boiling it in water for three hours.

3) The sample is rotor evaporated to dryness and then re-dissolved in acetic acid.

4) The sample is then put through an ion-exchange column and the aspartic acid fractions separated from the rest of the eluent.

5) The aspartic acid is then put through a HPLC column linked to a computer which separates base line peaks from the higher peaks of the L and D enantiomers of aspartic acid, and gives a value for the L/D ratio:

Dr. David Whittaker and his colleagues have now looked at around 90 pre-molars removed from living people or 'fairly fresh bodies' whose age at death was accurately known. They have used these data to calibrate the L/D ratio with age as well as testing the principle. The researchers found that they could determine the age of the body to within one year in 100 per cent of the bodies that had died in the last six months.

Little did the French Huguenots who settled in Spitalfields, London, in the 1600s dream that their practice of recording the ages at death on coffin plates, would one day be part of a research project.

In a joint venture with the British Museum funded by the Science and Engineering Research Council David Whittaker and his research group were able to carry out the 'ultimate test' for their method. The experiment was carried out on 100 teeth taken from the 300 year old bodies, in which the teeth were analyzed for their L/D ratios.

Although the ages at death of the Huguenot bodies were accurately known from their coffin plates, the information was not revealed to David Whittaker and his group until they had come up with their own answers. These were correct to within nine years, which I suppose is quite good for 300 year-old skeletons.

Whittaker said that 'The L/D ratio gives a more accurate measure of age than any other method we now use. Unfortunately, it is technically more difficult and more expensive than the translucency measurements. But we are sure that it is going to replace this old method fairly soon and set a new standard for forensic dentistry all over the world.'

The researchers are now looking more critically at the decomposing action of bacteria in the first few months after death to try to improve the accuracy of the technique.



SMILE

Research Definition

"It is hoped that this study will stimulate more work in this field." means:-

This is a lousy research report, but so are the others which have been published in this field of research.

10 Millionth Chemical

The American Chemical Society's Chemical Abstract Service (CAS) has recorded the 10 millionth chemical substance in its computer-based register of chemical substances. The substance, which has the chemical name cis-(±)-4,6,7,8,8a,8b-hexahydro-6,6,8b-trimethyl-3H-naphtho (1,8-bc) furan, is an intermediate product in a series of chemical reactions.

Meetings

IADR, General Session and
AADR Annual Session
April 17th-21st 1991,
Acapulco, Mexico

IADR, General Session 1992,
Glasgow, UK.

AADR, Annual Session, March
11th-15th 1992,
Boston, Mass.

IADR, General Session and
AADR Annual Session
March 10th-14th 1993,
Chicago, Ill.

Science Dull and Uninteresting ?

According to Owen Hertzman, we, as a country, are desperately short of good, young, Canadian scientists. Science as a career is often perceived by the general public as dull, uninteresting, not rewarding and not respected. How can we turn this around?

We should, as a society, make sure that we train those among our young people who have the ability and inclination to pursue careers in physics, chemistry, biology, etc. In order to motivate them, we have to ensure that there are adequate educational resources for them while they are in high school, enough scholarships while they are in university for five to 10 years, and stimulating jobs when they leave. If we ignore this problem, we risk becoming a second-rate and quite poor country within a generation.
(from The Mail-Star, Wed., Aug 1, 1990)

Important Post for Fournier

Bob Fournier Associate Vice President (Research) has been elected Chair of the Canadian Council on Animal Care (CCAC).

American City to Pay for Animal Rights Out of Taxation

Control by the local community over the use of laboratory animals in research, is seen by some researchers as a major problem. The City of Cambridge in Massachusetts have created a precedent in the US by appointing a commissioner of laboratory animals. The commissioner will monitor experiments involving live animals and make unannounced inspections of laboratories to ensure that researchers observe the provisions of the Animal Welfare Act. The universities in Cambridge presently have "institutional animal care and use committees" (IACUCs) similar to Dalhousie which aim to ensure that animals are treated according to the federal rules. One member of each committee must be a lay person, unaffiliated with a scientific organisation. The influence of lay members has been questioned by attorney Steven Wise, head of Boston's Animal Defense League and a member of the city's expert commission. The new commissioner will have the power to veto nominations for this seat on the IACUCs of the universities.

According to Steven Wise "The ordinance breaks important ground because it

demonstrates control by the local community over laboratory animals." However, Dr. Douglas Kelly, vice president for biomedical research at the Association of American Medical College, says: "It appears to be an unwarranted intrusion into the affairs of an institution that is already adequately covered."

Dr. John Moses, Chairman of the MIT's Animal Care Committee, has more confidence in the new system. He expects that it will enhance the accountability of the animal care committees. "We (at the MIT) have probably been remiss in the past," Moses says. "Here is someone who can look at us with a critical eye who does not represent scientists or animal rights groups."

Stuart Wiles, a practising veterinarian for 30 years, has been appointed by the City of Cambridge as animal protector. According to Wiles, the universities have finally accepted his role after earlier expressing "some misgivings." As for the animal welfare groups, Wiles predicts that although some will be satisfied, others will never be content.

The appointment of the first city commissioner of laboratory animals is very significant, since Cambridge is one of the major areas of

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scientific excellence in the US, with Harvard University and the Massachusetts Institute of Technology within its boundaries.

Cambridge's city council acquired a reputation for challenging Harvard, MIT and various research companies some 15 years ago. At the time city councillors attempted to limit research involving recombinant DNA, they claimed that such procedures posed unknown risks. The Cambridge City council's crusade for animals has been assisted by the Cambridge Committee for Responsible Research (CCRR).

The CCRR claimed three years ago that local laboratories were systematically abusing animals. A committee of experts set up by the city in 1988 investigated the claims and reported no serious abuses. However, the city council nevertheless passed an ordinance creating the post of commissioner.

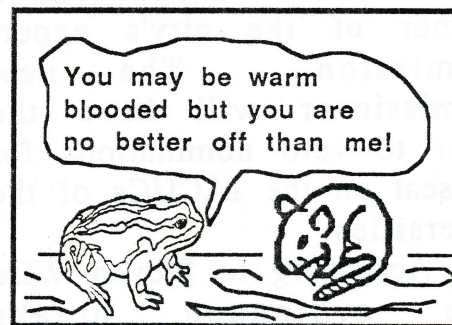
The director of CCRR, has said that local groups in Illinois, Vermont, and elsewhere in Massachusetts are also preparing to follow the lead of the City of Cambridge and are aiming to develop regulations to appoint a city commissioner of laboratory animals

The ordinance extends its rules for humane treatment of laboratory animals to

companies. Until the new ordinance, only universities receiving government money were covered by the rules. The companies include Arthur D. Little, Advanced Magnetic, Repligen, Biogen, and many others that have been drawn to Cambridge by the city's flourishing community of academic scientists.

The ordinance also covers rats and mice, which account for 90 per cent of the experiments on animals. This move is significant because scientist and animal welfare activists in the US are currently debating what protection rats and mice enjoy under the provisions of the Animal Welfare Act.

The act applies to warm-blooded animals: therefore, in theory, it applies to rats and mice. In practice, however, government agencies have not interpreted the act as covering rats and mice, mainly because they do not have the capacity to oversee the act if these animals are included



Animal Rights in Canada

About 2 million animals are involved in research in Canada every year. Some 85.5%, are involved in basic and applied research, a further 9.5% in mandatory testing, and 4.7% are used in teaching. Ninety percent of these 2 million animals are rats and mice, fish and fowl.



Most Canadians support the use of animals in research, which resulted in this country's development of the cardiac pace-maker, hypothermia techniques for open heart surgery, insulin, and a "Blue baby" operation. This endorsement is borne out, not only in opinion polls, but more importantly, by this support of our elected Parliament which provides funding for animal-based research, and the means of administering it.

Canada is attuned to world opinion in supporting animal research, as exemplified by the World Medical Association's Declaration of Helsinki (Venice, 1983), which requires that experimentation on animals precede human trials.

It should be noted that the International Guiding Principles for Biomedical Research Involving Animals, prepared by the Council of

International Organizations of Medical Sciences (CIOMS, 1985), states that:

"The advancement of biological knowledge and the development of improved means for the protection of the health and well-being both of man and animals require recourse to experimentation on intact, live animals of a wide variety of species."

In addition to use of animals in biomedical research, many animals are used yearly in agricultural and marine studies which improve Canada's food production, and help feed the world's hungry. As well, wildlife studies are important in maintaining an ecological balance, and in understanding animal behaviour.

Interestingly, animals have both benefited from, and been used in, research originally intended for humans. Dogs, for example, now receive pace-makers, artificial hips, insulin for their diabetes, and diagnosis by means of ultrasound and X-rays. The Ontario Veterinary College is building a radio-therapy unit to treat animal cancers.

Before, during and after any research takes place, the animals' welfare is the responsibility of the Dalhousie Universities Animal Care Committee (DUACC) and the principal investigator.

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(Animal Care Cont from P 10)
Both the Dalhousie University Animal Care Committee and the principal investigator are subject to the requirements of the Canadian Council on Animal Care (CCAC), a national, independent organization, founded in Ottawa 22 years ago. It is the CCAC's mandate to ensure ethical animal use and appropriate care.

CCAC is co-funded by Canada's two major granting agencies, MRC and NSERC. The CCAC comprises 17 member organizations involving scientist, educators, animal health technicians, and representatives of industry and the humane organizations.

The scientific and animal welfare communities are also included on all CCAC assessment panels which evaluate animal care and use in Canada's universities, as well as some community colleges, and government and commercial laboratories.

Extensive site visits are conducted at least every three years, plus additional, more frequent (often unannounced) visits. Assessments are based on CCAC's two-volume Guide to the Care and Use of Experimental Animals (1980, 1984). The Penalties for Non-compliance with the regulations can be very serious not only for an individual researcher but the whole institution. MRC and

NSERC who fund research involving animals require compliance with CCAC guidelines. Any institution which fails to follow the guidelines, may face sanctions imposed by MRC and NSERC which could lead to loss of all funding from both agencies.

Since the formalization of this policy in 1984, those institutions found in non-compliance have actively addressed their deficiencies, and it has not yet been necessary to have any research grant funds withdrawn.



An Early Animal Experiment

"People will inevitably associate me with my father, but I would not have anyone believe that I am trading on the name Edison. I would rather have you know me merely as the result of one of my father's earlier experiments." Charles Edison son of the inventor.

Time for a Kappa ?

