was removed from the adducts of the higher-boiling hydrocarbons either by holding the adducts at low pressure or by washing briefly with pentane (*ca.* 5 parts). Using an alternate method, a saturated solution of the ester was prepared in *n*-butyl alcohol and 5% by volume of *n*-hexadecane was added. Crystals appeared only on cooling the solution by 5–10°; these were treated as above, m.p. 68.0–69.5°.

The melting points of the isobutyl, *sec*-butyl, *t*-butyl and *n*-propyl 3,5-dinitrobenzoates and N-(*n*-butyl)-3,5-dinitrobenzamide were not affected by the presence of hydrocarbons; they presumably formed no adducts.

Octane Run.—A solution of *n*-butyl 3,5-dinitrobenzoate (84.5 mg., 0.315) mmole) was prepared in *n*-octane (2.0 g.) and the solution was allowed to evaporate (24°). After 13 hours, the total weight of the reactants had dropped to 87.8 mg.; the subsequent weight loss was rather small, 0.1 mg./day. The ratio by weight of ester to octane in the adduct was, therefore, 84.5/3.3. This is equivalent to 11 moles of ester per mole of octane.

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COMMUNICATIONS TO THE EDITOR

THE RECOVERY OF NORLEUCINE FROM CASEIN AFTER ADMINISTERING NORLEUCINE-3-C^{14} TO INTACT COWS[1]

Sir:

Norleucine has been reported to be a constituent of protein from brain tissue[2] but subsequent work demonstrated that DL-leucine, formed by racemization of L-leucine during protein hydrolysis, and not norleucine had actually been isolated in the original investigations.[3] More recent work has failed to confirm the presence of norleucine in protein.[4]

We injected intravenously two lactating cows with 3.6 millicuries (Cow #941, Trial I) and 3.3 millicuries (Cow #962, Trial II) of DL-norleucine-3-C^{14}, respectively. In Trial I, during 46 hours post-injection, 30% of the Carbon-14 was recovered in milk of which about one-third was present in casein. A casein sample prepared from the milk collected three hours post-injection was hydrolyzed, the amino acids separated on ion exchange resins, crystallized and assayed for C^{14} content according to methods described elsewhere.[5] The non-essential amino acids[6] had high C^{14} levels while the essential amino acids,[6] including leucine and isoleucine, did not contain significant amounts of C^{14}. However, the total C^{14} in the non-essential amino acids accounted for only 52% of the C^{14} in casein. A thorough recheck of all the effluent from the Dowex-50 column near the region of isoleucine–leucine elution revealed a small amount of material with high C^{14} activity that had emerged after leucine. The sample was identified as norleucine by paper chromatographic methods using various solvent systems (phenol, butanol–acetic acid, butanol–benzyl alcohol). We also have recovered and identified norleucine from casein prepared out of the milk collected ten hours after injection of norleucine-3-C^{14}.

We have only preliminary results from Trial II, but these appear to be much the same as results obtained in Trial I. In the casein collected three hours post-injection, the non-essential amino acids accounted for only 59.5% of the Carbon-14 in casein. Norleucine has been identified by C^{14}-measurements on a chromatogram of the casein hydrolysate but has not, as yet, been isolated from the Dowex-50 column.

The presence of norleucine in casein might result from adsorption or co-precipitation although our method for preparing the casein minimized this possibility. The casein was prepared according to our routine procedures by adjusting the *p*H to 4.6 with *N* HCl. The casein was filtered out, washed with water and then redissolved in *N* NH_4OH. This procedure was repeated and the casein, after the third precipitation, was washed with water, followed by alcohol and finally ether. The air dried casein sample was used for the C^{14} study.

To investigate the possibility of norleucine adsorption by casein, 40 microcuries of DL-norleucine-3-C^{14} was added to 1 liter of milk and after standing for 48 hours casein was prepared as described above. Casein samples were taken from the first, second and third precipitation for C^{14}-measurements. There was measurable activity only in the first precipitate and no significant C^{14} in the second or third precipitates. The *in vitro* trial was repeated with essentially the same results. These *in vitro* experiments demonstrate that our method for casein preparation effectively removes adsorbed (or co-precipitated) norleucine.

The results obtained with the intact cow presumably indicate incorporation of norleucine by peptide linkage. We are presently separating peptides from partial casein hydrolysates in an effort to isolate a peptide containing norleucine.

It was important to establish whether norleucine was a natural constituent of casein previously undetected because of low concentration. Control samples included five grams of casein prepared from the milk of one of our cows (Cow #962)[7] and another 5-g. sample of casein purchased from a commercial source. These samples were hydrolyzed, passed over Dowex-50 and the effluent

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from the "norleucine-region" concentrated. Aliquots of the concentrate were chromatographed in butanol–benzyl alcohol and failed to show any trace of norleucine. The sensitivity of this approach is limited but our results indicate that norleucine, if present, occurs in concentrations of less than 1.6 mg./100 g. casein. Norleucine from casein samples of Trial I were identified on the chromatogram both from the radioactivity and from the color reaction with ninhydrin spray. These samples contained considerably more norleucine than the maximum that could have been present in the control samples and from Carbon-14 measurements we have estimated that the minimum concentration of norleucine in the three hour casein sample of Trial I was 4.73 mg. of norleucine/100 g. casein.

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RECEIVED OCTOBER 7, 1955

SODIUM FLUOBORATE AS A FLUORINATING AGENT

Sir:

We treated triphenylchlorosilane with sodium fluoborate in acetone solution, in an attempt to prepare triphenylsilyl fluoborate, but the resulting product was triphenylfluorosilane which was obtained in fair yield. The reaction may be described by the equation

$$(C_6H_5)_3SiCl + NaBF_4 + (CH_3)_2CO \longrightarrow (C_6H_5)_3SiF + NaCl + (CH_3)_2CO{:}BF_3$$

This is believed to be the first reported use of sodium fluoborate as a direct fluorinating agent without the intervening preparation of a diazonium salt. It is the most convenient method known for replacing chlorine on silicon by a fluorine atom. No special apparatus is needed and acid solutions are avoided.

The Swarts reaction, using SbF_3,[1] has been most commonly used in carrying out these reactions; other methods employ anhydrous zinc fluoride,[2] or anhydrous hydrogen fluoride.[3] The most convenient method previously reported involved the reaction of the chlorocompounds with aqueous HF at 0°.[4]

Sodium fluoroborate was found to be insoluble in diethyl ether, petroleum ether and dioxane. It was slightly soluble in alcohol and soluble to the extent of 1.0 g. per 100 ml. in acetone.

Materials.—The triphenylchlorosilane was Dow–Corning purified grade. The sodium fluoborate was commercial grade which had been recrystallized once from water (m.p. 364–367°).

Preparation of Triphenylfluorosilane.—In a typical preparation, triphenylchlorosilane (6.32 g., 0.0215 mole) was dissolved in dry acetone and the solution poured into a clear acetone solution of sodium fluoborate (2.23 g., 0.0203 mole). A precipitate appeared almost immediately. After two hours the solution was filtered, with recovery of 76% of the calculated NaCl. The filtrate was concentrated, and on crystallization a trace of tetraphenylsilane, triphenylfluorosilane and an intractable tar were obtained in three different fractions.

On recrystallization, the triphenylfluorosilane (2.9 g., 54% yield) had a m.p. 59–60° (lit. value 61.5°).[5] A cryoscopic determination in benzene gave a molecular weight of 274 (calcd. 278). Qualitative tests confirmed the presence of silicon and fluorine and the absence of boron.

The tar contained material melting slightly above room temperature which gave a positive test for boron and fluorine but not for BF_4^- (by nitron test). The solid could not be easily isolated, as it decomposed on attempted recrystallization. This was assumed to be impure $(CH_3)_2CO{:}BF_3$.

In another reaction performed similarly, a 66% yield of triphenylfluorosilane was obtained. No attempt was made to determine optimum conditions for maximum yield.

One attempt was made to prepare triphenylsilyl fluoborate by passing BF_3 over a benzene solution of the fluorosilane, in the manner of Witschonke and Kraus,[6] but without success.

The generality of the fluorination is being investigated.

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RECEIVED OCTOBER 17, 1955

INCORPORATION OF ADENOSINE-5′-PHOSPHATE INTO RIBONUCLEIC ACID

Sir:

Since we have shown that cell-free preparations of pigeon liver that incorporate adenine into ribonucleic acid (RNA)[1] can also convert added adenine into adenosine-5′-phosphate (AMP),[2] it was pertinent to determine whether the mononucleotide was a precursor of the polynucleotide. Previous work indicated that mononucleotides were not as efficient as adenine in RNA formation by intact animals,[3] and that surviving tissue slices did not incorporate 5′ nucleotides into RNA.[4] A recent report, however, seems to implicate nucleoside-5′-diphosphates in RNA biosynthesis by extracts of micro-organisms.[5]

AMP labeled with C^{14} in the 4 and 6 positions of the adenine moiety was isolated from the pooled acid-soluble nucleotides derived from the viscera of mice that had been injected with adenine-4,6-

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